Mercury cycling in restored coastal wetlands

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I hereby declare that the work presented in this thesis is my own and has not been submitted elsewhere for any award.

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Abstract

Saltmarsh restoration is being implemented across Europe and North America yet, there is little understanding of the effects of de-embankment on Hg biogeochemical cycling. The aim of this thesis is to understand the controls upon Hg dynamics in coastal sites, with specific emphasis on the effects of ecosystem restoration on MeHg production. This is the first study in the UK to examine the effect of saltmarsh restoration on Hg methylation.

Field observations were used to assess broad-scale Hg dynamics and physico-chemical controls on MeHg production. A laboratory experiment was conducted to explore the short-term effects of saline inundation on MeHg production.

Recently de-embanked sites have lower MeHg concentrations, probably due to poor drainage and limited vegetation development. Physical sediment properties are less heterogeneous in restored sites, which are reflecting lower habitat and topographic heterogeneity. Previous land-use has a significant impact on physico-chemical sediment characteristics and these characteristics change over time to reflect saltmarsh development. There was evidence to show that it takes decades for restored sites to attain similar physico-chemical characteristics to their natural counterparts. This could have significant implications for wider biogeochemical cycling in restored saltmarshes, and long-term implications for the delivery of biogeochemical ecosystem services. This aspect of the research was completely novel, providing the first evidence of the spatial and temporal variation of Hg and MeHg concentrations in restored saltmarshes in the UK.

Laboratory experiments showed that MeHg production was greatest in fluctuating saline conditions (cumulative exposure of 1830 pg g^{-1}) compared to all other treatments. For example, the anoxic-saline treatment had a cumulative exposure of 460 pg g^{-1}. Findings indicated that peaks of MeHg could be produced immediately following tidal inundation and that MeHg could potentially increase over time as the site develops to a tidal regime more comparable to a natural saltmarsh. Previous

studies have indicated that permanently flooded soils produce high MeHg concentration however this is the first study to show that fluctuating saline conditions could produce a large pulse of MeHg, an important consideration for coastal managers.

Surface sediments in restored coastal wetlands appear to be areas of significant MeHg production. MeHg concentration was found to be well correlated with indicators of sulphate reducing bacteria (r=0.536, p<0.001), however most importantly, evidence was found for biogeochemical relationships with MeHg concentration, particularly the association of MeHg and indicators of iron reduction (r=0.561, p<0.001). Therefore, where MeHg is normally restricted by sulphide production, high levels of MeHg can be formed through other pathways. Coastal areas are not generally considered to be areas of concern for MeHg production because high chloride and sulphide concentrations have been shown to inhibit Hg methylation. However, this research shows that other pathways can also be responsible for Hg methylation (i.e. iron reduction) and therefore coastal sediment can be significant contributors to Hg methylation.

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Chapter 1: Introduction

1.1 Overview

Coastal development combined with sea-level rise has resulted in the loss and degradation of coastal wetlands worldwide (Atkinson *et al*. 2001). However, intertidal wetlands have a large conservation value and economic significance. They provide breeding and nursery areas for fish, grazing areas for livestock as well as attracting tourism, for example bird watchers. Coastal restoration implementation is therefore increasing in Europe and North America to improve the intertidal zone and maximise ecosystem functioning and services. Managed realignment (MR) is a type of coastal restoration that involves deliberately breaching an existing line of coastal defence to allow the tidal inundation of previously protected land (Andrews *et al*. 2006, Blackwell *et al*. 2004, Spencer *et al*. 2008). The technique is being increasingly implemented in Europe and North America due to high rates of sea level rise; in south-east England sea levels are predicted to reach 0.22 to 0.44 m above 1990 levels by the mid-2090s (Bindoff *et al*. 2007). The aim of MR is to restore saltmarshes so they function as both flood defences and ecological conservation areas (Macleod *et al*. 1999). However, recent research has suggested that restored sites may not revert back to fully functioning saltmarshes after inundation, a finding that has potential consequences for ecosystem functions such as vegetation growth, fish breeding and biogeochemical cycling (e.g., Wolters *et al*. 2005).

Mercury (Hg) contamination is a global environmental and human health issue. Although Hg occurs naturally in the environment at low levels, anthropogenic emissions over the last 100 years from waste incineration, chlorine manufacture, metal production and coal combustion (Environment Agency 2010) have resulted in even remote areas being Hg-contaminated at levels much higher than under natural conditions (Fitzgerald *et al*. 1998). In anaerobic conditions, inorganic mercury (Hg[II]) is converted to methylmercury (MeHg), a potent neurotoxin that biomagnifies to dangerous concentrations in food webs. The majority of MeHg production in coastal

wetlands is produced by *in situ* methylation of Hg[II] by sulphate-reducing bacteria (SRB) and iron-reducing bacteria (FeRB) (Fitzgerald *et al*. 2007, Mitchell and Gilmour 2008). SRB thrive in organic-rich, anaerobic sediments in both aquatic environments and terrestrial wetlands (Hall *et al*. 2008), especially in saline ecosystems where sulphate (SO_4^{2-}) is abundant (Andrews *et al*. 2006). Coastal ecosystems that produce large amounts of MeHg may lead to localised 'hot spots' in human exposure (Sunderland *et al*. 2006).

Saltmarsh restoration can result in substantial changes to the sediment's physicochemical characteristics and concerns have been raised where former agricultural land has undergone MR. Physical changes in the sediment structure has been reported and evidence of an over-consolidated horizon within the upper 10 cm of the sediment profile has been observed. Moisture content has been shown to increase in the newly deposited sediment (Garbutt *et al*. 2006, Spencer *et al*. 2008) and chemical changes in sediment properties have also been reported; a decrease in sediment pH after tidal inundation had been reintroduced (Blackwell *et al*. 2004). Changes in sediment chemistry such as pH can alter the partitioning, and hence mobility and bioavailability, of contaminants (Macleod *et al*. 1999). Increasing the mobility and bioavailability of Hg can result in an increase in Hg methylation and bioaccumulation into the food chain. This is particularly pertinent where MR takes place in urbanised estuaries that have historically received higher Hg inputs than rural estuaries due to the proximity of Hg contaminant sources. Coastal systems are recognised sinks for many terrestrially-derived contaminants, including Hg, and often contain large reservoirs of legacy Hg in the sediment profile (Hammerschmidt *et al*. 2004).

Waterlogged, anaerobic saline sediment with a low pH provides an ideal environment for Hg[II] to be methylated to MeHg (Sunderland *et al*. 2006), an important concern that has not been considered when implementing MR schemes. Numerous studies have recognised the lack of understanding of Hg biogeochemistry in estuarine and coastal ecosystems compared to terrestrial and freshwater environments (Sunderland

et al. 2006, Hammerschmidt *et al*. 2004, Mitchell and Gilmour 2008). There is a clear need for further research to be conducted into the role of estuarine environments in the Hg cycle. Furthermore, even fewer studies have examined Hg bioavailability on restored coastal wetlands (the South Bay Salt Pond Restoration Project in North San Francisco Bay estuary is one of the few examples (Grenier *et al*. 2010)) and how the changes in sediment physico-chemical characteristics that occur post-realignment might affect Hg bioavailability.

1.2 Evidence for the importance of mercury cycling in coastal wetlands

In anaerobic conditions, Hg[II] can be converted to MeHg and even low levels of MeHg in surface water can bioaccumulate to high MeHg concentrations in fish (Morel *et al*. 1998). Bioaccumulation is clearly evident in aquatic ecosystems where Hg concentrations of plankton have been measured 10,000 times higher than in the surrounding water (Krabbenhoft 1996). Methylmercury has a high affinity for fatty tissues and so bioaccumulates and biomagnifies more readily than other species of mercury (Ravichandran 2004). MeHg concentration in fish from even remote areas have often been close to and sometimes exceeded the level deemed safe for human consumption (0.5-1ppm) (Morel *et al*. 1998). Coastal ecosystems that produce large amounts of MeHg may therefore lead to an increase in human exposure as well as negative ecosystem effects such as a decrease in reproductive success in fish and fish-eating birds (Sunderland *et al*. 2006). The MeHg contamination of fish has led to the creation of consumption guidelines and health advisories worldwide. The primary source of MeHg production is within the sediment, through biotic mechanisms. Therefore, increasing wetland area through restoration has the potential to produce hotspots for MeHg production (Marvin-DiPasquale and Cox 2007). Research at the South Bay Salt Pond Restoration Project in North San Francisco found poor correlation between sediment THg and biota MeHg suggesting that other wetland processes control MeHg production rather than THg concentrations (Yee *et al*. 2005). Further research into Hg cycling, and specifically into the sediment characteristics in restored

coastal wetlands is therefore highly topical, and is required to understand the high level of complexity of Hg cycling in coastal wetlands. This PhD aims to understand the controls upon Hg dynamics in coastal sites, with specific emphasis on the effects of ecosystem restoration on MeHg production.

Chapter 2: Literature Review

2.1 Saltmarshes

Saltmarshes are intertidal grasslands that form in wave-sheltered areas allowing the deposition of fine sediment which is stabilised by vegetation (Boorman 2003, Hall *et al*. 2008). Saltmarshes are periodically inundated by sea water which infiltrates the saltmarsh sediment during high tide and drains at low tide (Boorman 2003). Vegetation will vary spatially from the seaward to landward edge of the saltmarsh. Plant species found at the seaward limit will have higher tolerance to tidal inundation and salinity, whereas species composition at the upper limit is determined by competition with other plant species less tolerant of saline conditions (Boorman 2003). Once vegetation is established, sediment accretion rates rapidly increase and the elevation of the marsh surface is raised. Vegetation varies with individual species' responses to the elevation gradient (Boorman 2003).

Saltmarshes are also among the most biologically-productive ecosystems in the world (up to 3900 g C m^{-2} yr^{-1}) (Mitsch and Gosselink 2000) and provide valuable ecosystem services and functions to humans, including coastal protection, water purification (nutrient and pollution uptake), carbon sequestration, improving water quality, tourism, recreation, education, and research (Barbier *et al*. 2011, Andrews *et al*. 2006, Lillebø *et al*. 2010). The carbon sequestered in saltmarsh sediments is generally stored in anoxic environments resulting in the slow decay of biomass in the form of peat. Therefore, carbon is transferred into the long-term carbon cycle (1000 years), and is removed from the atmosphere, potentially reducing global warming (Barbier *et al*. 2011). Millennium Ecosystem Assessment (2005) estimated that, globally, coastal ecosystems provide US$15 trillion worth of services annually; nutrient cycling (storage, internal cycling, processing, and acquisition of nutrients) is their most valuable environmental service. Filtration of contaminants and nutrients improves the quality of

surface waters and indirectly improves the breeding success of local fisheries (Montalto *et al*. 2006). For example, secondary production derived from coastal marshes in the Gulf of Mexico, accounts for up to 66 % of shrimp (Zimmerman *et al*. 2002) and therefore, good water quality is important to maintain successful shrimp breeding. In addition , coastal wetlands also have the potential to provide marketable goods such as shellfish, samphire and wildfowl, and provide recreational sports like fishing and waterfowl hunting (Ledoux *et al*. 2005). Saltmarshes have a high wildlife and nature conservation value, and provide spawning sites and nursery areas for fish, habitat areas for a wide range of bird species (Boorman 2003), and unique plant and microbial communities.

Saltmarsh sediments undergo regular tidal inundation. Drainage patterns (controlled by height relative to tidal frame, topography, sediment type, distance to marsh/creek edges) across the marsh can influence the spatial variability of sediment saturation which in turn influences oxidation state of the sediment, the microbial communities and the rates of mineralisation in these ecosystems (Montalto *et al*. 2006). Saltmarsh sediments have high clay, silt and organic matter content as well as a relatively flat topography, which slows the drainage of saturated sediments after tidal inundation.

For over 300 years, UK coastal and estuarine management strategies have been dominated by land reclamation and flood protection, primarily through the drainage of wetland systems and construction of 'hard' engineering defences (Andrews *et al*. 2006). However, these strategies are not sustainable and may have actually exacerbated saltmarsh loss. Current coast defence techniques are being increasingly questioned due to concerns over sea-level rise and the integrity of existing sea defences, many of which have reached the end of their usable lifespan (Hughes and Paramor 2004). An estimated rise in relative sea level (RSL) of 1.5 mm per year in the south-east of England (a combination of climate change and isostatic readjustment) has meant that 40 ha of saltmarshes are still being eroded each year due to coastal squeeze although this is debated in the literature (Hughes and Paramor 2004, Morris *et al*. 2004, Boorman 2003, Hughes 2004). An increase in RSL causes the landward

migration of the vegetation zones within a saltmarsh (Hughes and Paramor 2004). However, if this upward progression is restricted and held stationary by hold-the-line strategies (e.g., sea wall), then as the seaward edge of the saltmarsh moves inland there is a subsequent loss of habitat. The vegetation cannot survive in a zone of more regular tidal inundation and increased salinity and the marsh is said to be 'squeezed' (Hughes 2004, Pethick 2002). The cost of maintaining and upgrading existing sea defences coupled with the loss of saltmarshes has, in recent years, caused the focus of coastal management to shift towards alternative strategies, such as managed realignment, that allow the management of coastal environments in a more sustainable way (Andrews *et al*. 2006).

2.2 Managed realignment

Managed realignment, also known as de-embankment, set-back and coastal retreat, is the deliberate breaching of the existing coastal defence to allow the inundation of previously protected land (Andrews *et al*. 2006, Blackwell *et al*. 2004, Spencer *et al*. 2008). The line of defence is relocated landwards so that an area of land can be flooded and a saltmarsh can develop (French 2006). The aim of MR is to restore saltmarshes so they function as both flood defences and ecological conservation areas (Macleod *et al*. 1999). By returning land to the sea allows saltmarsh and intertidal mudflats to develop which has several perceived benefits to coastal managers. For example, managed realignment is thought to increase wave attenuation as well as reduce sea-level rise (locally) due to increase tidal volume (French 2006). Other benefits include increasing species richness and biodiversity, although there is much debate on how successful managed realignment has been in achieving these aims (Mossman *et al.* 2012).

MR is becoming an attractive alternative to the current hold-the-line policy (constructing hard sea defences such as concrete sea walls so the coastline remains stationary) because of the increasingly high cost associated with maintaining hard sea defences as well as the other desirable functions saltmarshes provide (Garbutt *et al*. 2006). Cox *et al*. (2006) highlight how MR sites, coupled with dike elevation, can be

used as flood control areas and an estuary can be protected against flooding with a return period of 4000 years, without the implementation of costly defences like seawalls or flood barriers, again offering considerable savings.

The importance of maintaining saltmarsh biodiversity is now recognised in law under the European Union Habitats Directive (Pethick 2002), which specifies a no-net-loss policy within large designated areas (Blackwell *et al*. 2004). Introduced in 1994, the Habitats Directives prohibits any development that could threaten habitat diversity within designated special areas of conservation (Pethick 2002); more than 80 % of current UK saltmarshes are now covered by one or more national or international conservation designations (Hughes and Paramor 2004). The UK is committed to maintaining the existing extent of saltmarsh habitat and restoring the total area of saltmarsh to 1992 levels (English Nature 1999). As a result, there has been a recent shift to a more sustainable approach to coastal management that includes alternative strategies, such as managed realignment (MR) with subsequent saltmarsh development, which are more economically viable. Creating coastal wetlands through MR complies with these policies and fulfils the UK Government's commitment to them.

Managed realignment is increasingly being implemented in the UK, with over 40 sites having been commissioned since 1991, predominantly in the south-east of England. MR has been implemented in other countries (e.g., United States) since the 1970s. For example, the State of California and U.S. Government purchased 6,475 hectares of industrial salt-production ponds in 2003 from the Cargill Salt Company with a primary goal of wetland restoration (Marvin-DiPasquale and Cox 2007). However, despite the perceived benefits of MR, concerns have been expressed over the technique being promoted without a full understanding of the implications for both the MR sites themselves and the wider environment (Spencer *et al*. 2008).

Restored sites have a long management history from being reclaimed and drained for agricultural use, to a return to tidal hydrology following de-embankment. These changes have had a large impact in the sediments' physical and chemical

characteristics. When saltmarsh is initially reclaimed from the sea, the sediment profile goes through many geophysical changes. Initially, the sediment is partly waterlogged, saline, and rich in organic material. The sediment desalinates after a period of flushing with rainwater and drainage of these saltmarsh sediments, causing the clay fraction to become dispersed (Crooks *et al*. 2002). The dispersion of clay minerals is enhanced in south-east England where sediments are fine-grained and depleted in calcium carbonate (typically less than 1 %) (Crooks *et al*. 2002). The soil fabric collapses following de-sodification of the marsh soil and the pore spaces become smaller. The elevation of the marsh is decreased in comparison with the surrounding area due to this consolidation, as well as the physical processes associated with agricultural activities (Wolters *et al*. 2005) and organic matter mineralisation. This transition from a marine- to fresh-water environment results in the formation of a low permeability, over-consolidated soil horizon and changes to the soil structure are permanent (Crooks and Pye 2000, Crooks *et al*. 2002).

There is evidence that these pre-breach over-consolidated soil horizons, with low hydraulic conductivity, act as a barrier to vertical water movement impeding sub-surface drainage and tidal flushing in these sediments (Garbutt *et al*. 2006, Tempest *et al*. 2014, Spencer *et al*. 2008). This results in waterlogged sediments with low redox potential, low bulk density and low resistance to erosion and this has been responsible with poor species composition at some MR sites (Mossman *et al*. 2012). Changes in soil density, porosity and organic content are a lot more apparent in sites that were used for arable farming and less obvious in sites that remained unploughed (French 2006). MR is therefore being promoted without a full understanding of the environment that is being created and MR sites do not always return to natural saltmarsh conditions in terms of sediment properties, potentially having significant impacts on hydrology which could hinder vegetation establishment and biogeochemical cycling.

The biogeochemistry of MR sediment has been shown to be significantly different to that of a natural saltmarsh (Macleod *et al*. 1999). Inundation with seawater and microbial decomposition of organic matter causes strong vertical biogeochemical

gradients to form within the restored saltmarsh sediment profile. MacLeod *et al*. (1999) reported that redox potential of a MR site had decreased from values indicating oxic soils, to values lower than those usually reported for mudflat sediments. The change in redox can potentially alter the biogeochemical cycling on contaminants into the sediment by increasing their mobility and bioavailability.

2.3 The global mercury cycle

Mercury occurs naturally at low levels in the environment however anthropogenic emissions over the last 100 years from waste incineration, chlorine manufacture, metal production and coal combustion (Environment Environment Agency 2010) have resulted in even remote areas thousands of miles away from point sources becoming Hg-contaminated (Fitzgerald *et al*. 1998). The three principal species of Hg are elemental mercury (Hg^0), inorganic mercury (Hg[II]) and methylmercury (MeHg). Hg^0 has low solubility and volatilises easily, allowing it to leave the aquatic environment to the atmosphere in the vapour phase. Surface waters are saturated with $Hg^0(aq)$ and therefore there is a flux from the water to the atmosphere (Fitzgerald *et al*. 2007). Approximately 95 % of atmospheric Hg is in the form of $Hg^0(g)$ which can reside in the atmosphere for 1.2 to 1.7 years (Shia *et al*. 1999, Rasmussen 1994) and travel long distances before it is photooxidised to Hg[II] (Morel *et al*. 1998). Inorganic Hg is readily available and therefore quickly binds with particles or dissolves in water, and is deposited via dry or wet deposition respectively (see Figure 2.1).

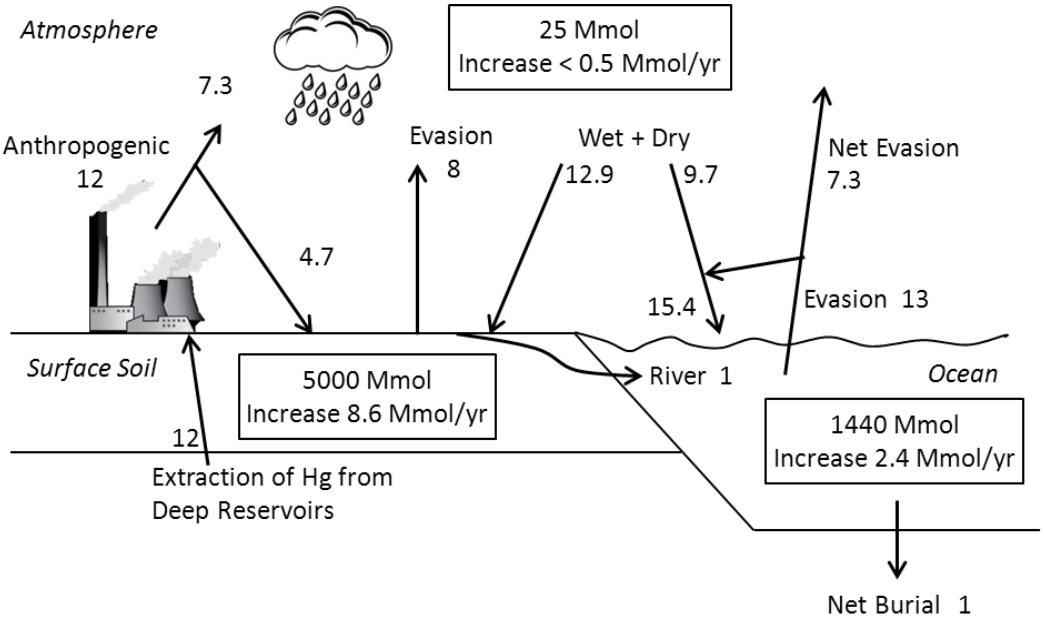

Figure 2.1. The global mercury cycle. All fluxes are in Mmol yr^{-1} (Mason and Sheu 2002).

2.4 Sources of mercury to the coastal zone

The industrial use of Hg as well as widespread agricultural application of organomercurials has resulted in the wide spread contamination of surface waters and sediments (Ullrich *et al.* 2001). Coastal sediments are a repository for natural and pollution-derived Hg and can act as both sinks and sources of Hg depending on the prevailing physical, chemical and biological conditions (Hammerschmidt and Fitzgerald 2004). The main source of Hg to tidal wetlands is either from legacy Hg already deposited in historic sediments or Hg transported into the wetland from sources external to the estuary (Davis *et al*. 2003).

Mercury stored in the sediment can be remobilised during tidal inundation as well as by other types of disturbance, for example bioturbation (Hammerschmidt *et al*. 2004). Hg compounds can be taken up by biota, released to the atmosphere, sequestered and

buried in sediment, transported with particulate matter to other locations, or released from the sediment into overlying waters (Ullrich *et al*. 2001). Wetlands can also be an important source of indirect Hg emissions and the formation of volatile Hg species can be on the same order of magnitude as industrial emissions (Wallschläger *et al*. 2000). A large seasonal source of atmospheric Hg could also come from agricultural land during tillage of fields within close proximity to wetlands (Bash and Miller 2007).

2.5 Mercury biogeochemical cycling in the coastal zone

Saltmarsh sediments are generally rich in organic matter and contain relatively high clay content. Therefore, sediments contain high moisture content and oxygen is utilised rapidly within a few millimetres of the surface (Kostka *et al*. 2002). Salinity also promotes waterlogging because it de-flocculates the clay particles so that structureless sediment with low hydraulic conductivity is produced (Long and Mason 1983).

Redox potential decreases with sediment depth due to the utilisation of oxygen by microbes within the sediment and most organic matter remineralisation occurs under anoxic conditions (Kostka *et al*. 2002). Once consumed, the next significant alternative electron acceptors (NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2) are utilised by microbes creating a vertical biogeochemical gradient within the sediment (see Figure 2.2; Klüpfel *et al*. 2014). Consequently, saltmarsh sediments are characterised by redox-stratified sediments. Any metals, such as Hg, complexed to these alternative electron acceptors are released into porewater (Otero *et al*. 2009). Metals in their dissolved phase are significantly more mobile and bioavailable than solid-phase metals. Furthermore, sulphate reducing bacteria (SRB) are responsible for up to 90 % of the organic carbon mineralisation in saltmarsh sediments and are also the key reducing bacteria responsible for methylmercury (MeHg) production; although other reducing bacteria such as iron reducing bacteria (FeRB) can also be important (Mitchell and Gilmour 2008). Therefore, restoring coastal wetlands has the potential to create significant sources of MeHg and have a substantial impact on the surrounding ecosystem.

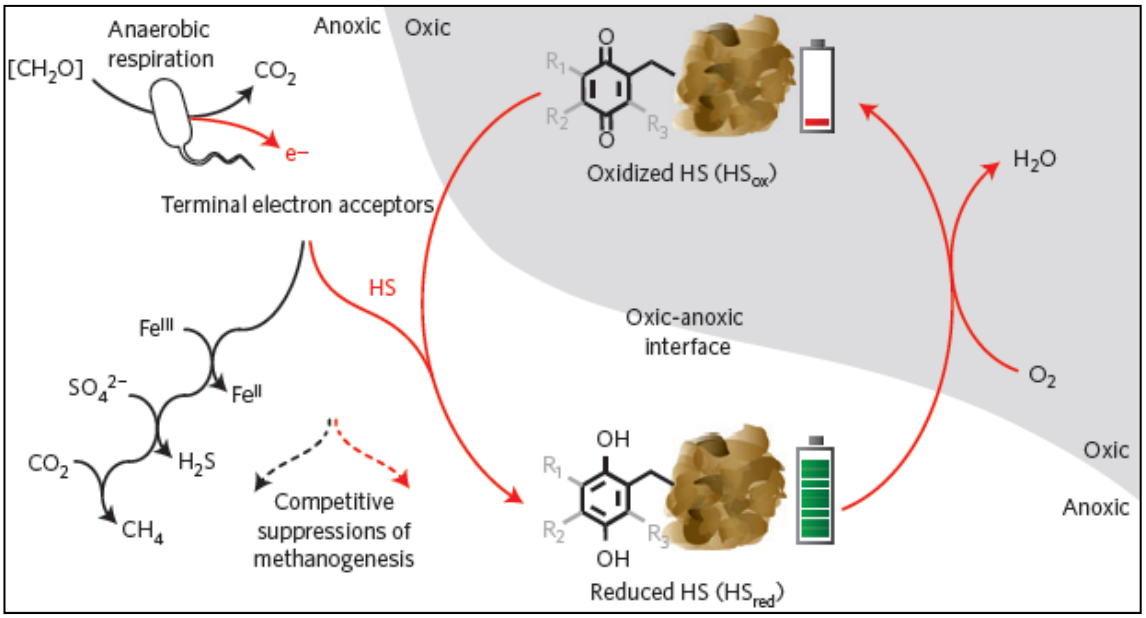

Figure 2.2. Microbial reduction of HS under anoxic conditions and subsequent HS re-oxidation by O_2 under oxic conditions (taken from Klüpfel *et al*. 2014).

A conceptual model of Hg cycling in tidal wetlands is shown in Figure 2.3. Mercury occurs in three valence states (0, +1, and +2) and the reactions of these species with inorganic and organic ligands determines the mobility and bioavailability of Hg in aquatic ecosystems, as well as the potential for Hg methylation (Ullrich *et al*. 2001). The main Hg species found in the coastal zone are complexes of inorganic mercury (Hg[II]) with various ligands (such as organic matter and sulphur), and organic Hg (methylmercury; MeHg). Dimethylmercury (DMH), another form of organic mercury, is generally only found in deep ocean waters. DMH only forms in the low oxygen region because it is unstable and in other areas it will demethylate to MeHg (Mason and Fitzgerald, 1993).

Inorganic Hg[II] and methylmercury (MeHg) can be reduced to Hg^0 by various mechanisms, including reduction by microorganisms, abiotic reduction by humic substances, decomposition of methylmercury and photoreduction (Ullrich *et al*. 2001). Photoreduction rather than microbial reduction is the principle mechanism responsible

for MeHg and Hg reduction, although the efficiency of photoreduction is controlled by the concentration of Hg[II] as well as the wavelength and intensity of radiation (Morel *et al*. 1998). Unicellular microbial reduction is the principal mechanism of Hg[II] reduction in contaminated environments. The MerA reductase is a metal resistance mechanism present in bacteria that reduces Hg[II] to Hg^0 (Morel *et al*. 1998). Surface waters are supersaturated with Hg^0 compared to the atmosphere and so elemental Hg is constantly lost from the aquatic environment via diffusion to the atmosphere.

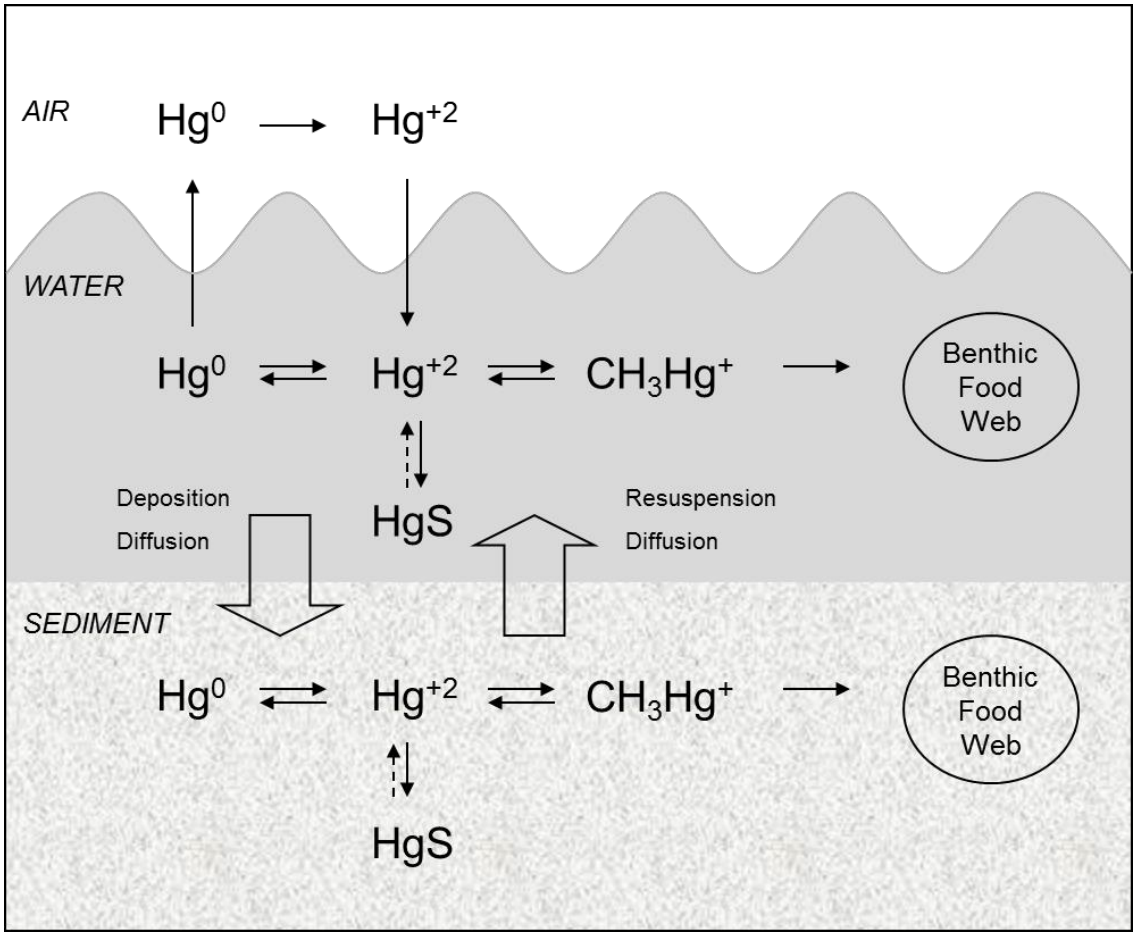

Figure 2.3. Mercury cycle in tidal wetlands (Davis *et al*. 2003)

Methylmercury is formed when dissolved Hg[II] crosses the lipid membrane that surrounds the unicellular methylating bacteria, either by diffusion or active uptake. The Hg[II] is methylated through the addition of an alkyl anion group (such as CH_3^-) (Benoit *et al*. 1999, Morel *et al*. 1998). THg concentration however, is not always a

significant control on Hg methylation and studies have often found poor correlation between THg and MeHg concentrations (Heim *et al*. 2007, Sunderland *et al*. 2004). A lack of correlation between THg and MeHg suggests that THg concentration is not the main factor limiting MeHg concentrations and Hg methylation depends more on Hg[II] bioavailability and the microbial activity that convert Hg[II] to MeHg. Therefore, other environmental factors are important in mediating Hg methylation, such as partition coefficients of Hg, sulphide concentrations and organic matter content (Yee *et al*. 2005, Grenier *et al*. 2010, Benoit *et al*. 2003, Hsu-Kim *et al*. 2013).

Inorganic Hg[II] is most susceptible to methylation when it is in the dissolved phase and least susceptible when it is in the particulate state (Davis *et al*. 2003) because the particle is too large to pass through the cell membrane. Therefore, dissolved-solid phase partitioning reactions are important for regulating Hg bioavailability (Krabbenhoft *et al*. 2005).

The distribution coefficient (K_D) of Hg is defined as the ratio of the particulate concentration (P) to the dissolved concentration (C):

$$K_D = \frac{P}{C}$$

The K_D effects the bioavailability and transport of Hg in the aquatic environment (Turner *et al*. 2001, Turner 1996). For example, Hg[II] in the solid phase has a high affinity for organic matter and it is transported through a variety of mechanisms with organic matter in the environment. However, when the organic matter is mineralised, Hg[II] is released into the aqueous phase and becomes more bioavailable to bacteria for methylation.

Environmental conditions such as organic matter content, redox status, sulphur, iron and pH, control the partitioning and bioavailability of Hg in coastal wetlands, each of which will be discussed below.

Clay content can influence the concentration and bioavailability of metals in a soil. Clay minerals have a large surface area and exchange capacity compared to other minerals. Metal concentrations an order of magnitude higher have been reported for soils dominated by secondary minerals (e.g. clay) rather than primary minerals (e.g. quartz) (Zhong and Wang 2008). However, although metal concentration may increase, the bioavailability of that metal decreases because of the strong bond between Hg-organic matter and clay. Removing the clay content altogether has been shown to increase the extractable Hg from a soil from 30 % to 55 % (Kongchum *et al*. 2011).

2.5.1 Organic matter content

Adsorption of Hg[II] to organic matter has been identified as an important mechanism that facilitates the transport, as well as the bioavailability of Hg within the aquatic environment (Bryan and Langston 1992). Sediment organic matter can describe 80% of the spatial distribution of both Hg[II] and MeHg (Hammerschmidt *et al*. 2004). However, organic matter has a complex relationship with Hg given that it can either promote methylation by providing a labile carbon source and stimulating microbial growth or reduce the bioavailability of Hg[II] through complexation. DOC can also inhibit Hg and MeHg reduction to Hg^0 because it scavenges the UV radiation before it can photoreduce Hg[II]. Therefore, higher photoreduction rates are observed in low-DOC lakes (Morel *et al*. 1998).

Complexation of Hg[II] to organic matter creates molecules that are too large to pass over the cell membranes of the methylating bacteria and therefore decreases the bioavailability of Hg and hence its bioaccumulation in the food web (Haitzer *et al*. 2003). Turner *et al*. (2001) found that the sorption of Hg to sediment decreased by 1-2 orders of magnitude after the particulate organic matter fraction of the sediment had been removed. Hg[II] forms strong complexes with the acid sites in organic matter and preferentially binds to reduced sulphur (-S) or thiol (-SH) functional groups (Ravichandran 2004). Mercury, like other heavy metals, is a ‘soft’ metal cation and is characterized by polarised electrons in the outer shell giving it a positive charge. Soft metals, like Hg, prefer ‘soft’ binding sites on organic matter, like thiol groups over

ligands containing oxygen (Ravichandran 2004). The stability constant (a measure of the strength of interaction between molecules) for Hg[II] complexation with sulphate is $10^{1.3}$, whereas, the stability constant for Hg[II] complexation with sulphide is $10^{52.4}$ (Ravichandran 2004). Therefore, Hg[II] is less bioavailable to methylating bacteria when complexed to sulphide. The sulphur-containing functional groups only make up a minor fraction of organic matter, ranging from 0.5% to 2.0% by weight and an even smaller fraction of these groups exist in a reduced state. However, organic matter is usually at concentrations much higher than Hg so there is usually more than sufficient reduced reaction sites within organic matter to bind with the available Hg[II] (Haitzer *et al*. 2002, Haitzer *et al*. 2003).

Labile organic matter can also stimulate microbial activity. When organic material is mineralised the Hg associated with the organic matter is released into the surface water with dissolved organic carbon (DOM). Strong correlations between dissolved total-Hg and DOM have been observed in wetland ecosystems (Hall *et al*. 2008) because DOC is the primary ligand of inorganic Hg in oxic surface waters (Yee *et al*. 2005).

Seasonality has a large control on Hg methylation rates because of its control on organic matter production, with highest MeHg concentrations being reported in summer (Heyes at al. 2004, Choe *et al*. 2004). The minimum MeHg concentration occurs before the spring 'bloom'. Plankton blooms increase the organic matter content of the sediment providing a source of labile carbon for microbes to utilise. This consequently decreases the redox status of the sediment and increases the reduction of SO_4^{2-} and Fe oxyhydroxides (see section 2.5.2 and 2.5.3) and potentially methylating any Hg also bound to these complexes. Also, during periods of intensive growth (spring and summer), when respiration is greatest, microbial activity in the live root zone will also be greatest. Plant roots and benthic organisms can introduce oxygen into the sediment, increasing microbial activity and therefore increasing MeHg concentrations.

2.5.2 Iron

In the absence of O_2, microorganisms may use other electron acceptors, such as Mn and Fe oxyhydroxides. The reduction of Mn[IV] and Fe [III] to Mn[II] and Fe [II] respectively at the oxic/anoxic boundary is associated with the increase solubility of Fe and Mn as well as other metals, such as Hg, bound the surface of the oxides (Otero *et al*. 2009). Inorganic Hg is released into solution and is more bioavailable than sediment phase Hg. Previous studies suggested that Hg[II] methylation rates are often more strongly related to total organic carbon mineralisation rates than sulphate reduction rates suggesting that other pathways of organic carbon mineralisation may be important in MeHg production (Mitchell and Gilmour 2008, Bloom *et al*. 1999, Hammerschmidt *et al*. 2004). Kostka *et al*. (2002) reported that Fe[III] reduction is the dominant microbial respiration path in bioturbated or vegetated saltmarsh sediments, whereas sulphate reduction was more pronounced in sediments without macrofauna or macrophytes, an imported consideration in vegetated saltmarshes. Oxygen introduced by plant roots stimulates Fe cycling in saltmarsh soils by oxidising reduced Fe (Fe[II]) and increases MeHg production through the increased activity of FeRB (Yee *et al*. 2005, Kostka *et al*. 2002).

2.5.3 Redox status and sulphur cycling

Methylmercury is mainly formed through biotic processes and is thought to be the accidental by-product of sulphate reduction in sulphate reducing bacteria (SRB) (Benoit *et al*. 2003). SRB are widely accepted as the key methylators of Hg[II] at the oxic/anoxic interface, although other reducing bacteria such as iron reducing bacteria (FeRB) can also be important (Kerin *et al*. 2006, Mitchell and Gilmour 2008). Therefore a reducing environment needs to be established before MeHg will be produced and methylation is generally greatest at the oxic/anoxic boundary. MeHg is formed mainly through the formation of neutral Hg-S complexes which diffuses over the SRB membrane. Therefore, sulphur concentrations, sulphate concentrations (an electron accepter for SRB) and labile carbon (an electron donor for SRB) are important variables controlling SRB concentrations and consequently methylation rates (Lambertsson and Nilsson

2006). MeHg can also be formed through less common abiotic processes, for example photochemical reactions involving acetate or humic acids (Morel *et al*. 1998).

SRB thrive in organic-rich, anaerobic sediments in aquatic environments (Hall *et al*. 2008), especially in saline ecosystems where sulphate is abundant (Andrews *et al*. 2006)**.** SRB are responsible for 50-90 % of the organic carbon mineralisation in coastal sediments (Fitzgerald *et al*. 2007). Given its largely bacterial source, MeHg production is therefore ultimately controlled by many of the same factors that determine microbial community dynamics (Sunderland *et al*. 2006), as well as the availability of pre-existing Hg[II] for methylation by those bacteria.

Hg methylation has generally been found to be lower in estuarine sediments compared to freshwater sediments because high sulphide levels (a by-product of SRB activity) inhibit Hg methylation rates by either creating Hg-S complexes that are too large to diffuse over the SRB membrane or by removing inorganic Hg from the solution and precipitating it into the solid phase as HgS (Benoit *et al*. 2003). The availability of Hg[II] is a prerequisite for Hg methylation.

The Hg-S complex is controlled by the dissolved sulphide concentration, which in turn is controlled by the redox status of the sediment. Dissolved sulphide outcompetes other ligands for complexation with Hg and increases the dissolved Hg concentration potentially making it more bioavailable (Morel *et al*. 1998, Benoit *et al*. 1999). However, high sulphide levels make $HgHS_2^-$ likely to be the major Hg-S complex which is less bioavailable than HgS^0 to methylating bacteria (Fitzgerald *et al*. 2007). Maximum Hg methylation rates will be observed in sediment with neutral Hg complexes such as HgS^0, and will be less evident in highly reducing sediments where larger negative complexes such as HS_2^- and $Hg(S_x)_2^{2-}$ will be more prevalent (Figure 2.4; Fitzgerald *et al*. 2007, Compeau and Bartha 1987). Therefore, Hg methylation rates will tend to increase with sulphide concentration up to approximately 1.02 mg L^{-1} but then start to decrease at higher levels (Morel *et al*. 1998, Craig and Moreton 1983b).

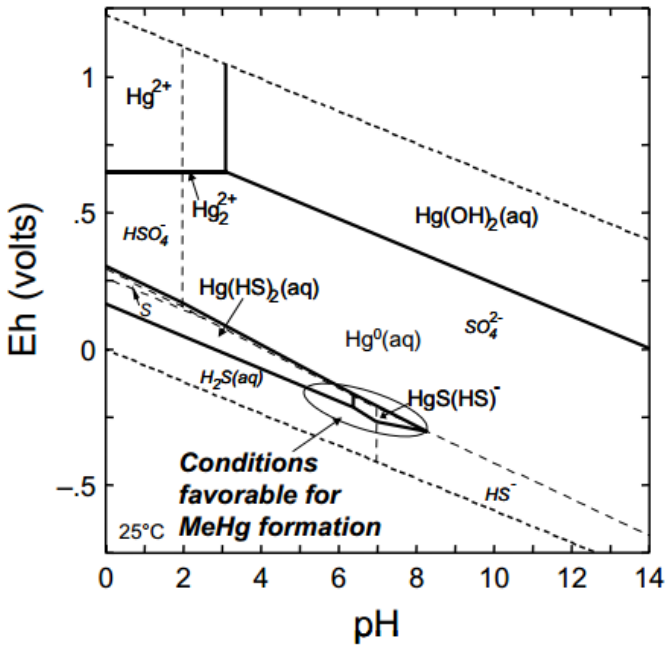

Figure 2.4. Eh-pH predominance diagram for Hg in the presence of sulphur at 25 °C (taken from Reeder *et al*. 2006).

Key indicators can be measured to assess SRB activity. These include porewater sulphide, sediment acid-volatile reduced sulphur (AVS) and chromium reducible sulphur (CRS). AVS (FeS) and CRS (FeS_2) can be regarded as temporary sinks for sulphides. Sulphide concentration in porewater can be low due to the rapid precipitation of AVS (FeS) in the presence of abundant dissolved Fe[II], whereas low dissolved Fe[II] concentrations can accelerate the diffusion of sulphides towards the oxic/anoxic interface where they are oxidised and recycled (Billon *et al*. 2001b). CRS concentrations are less significant because this pool is likely recalcitrant and contributes less significantly to Fe or S cycling (Mitchell and Gilmour 2008).

Mercury methylation also increases with sulphate concentration up to levels of approximately 19-48 mg L^{-1} in anoxic sediments because it stimulates SRB activity, after which MeHg production decreases (Compeau and Bartha 1987)**.** High sulphate concentrations have been shown, through numerical modelling and laboratory experiments, to only inhibit Hg methylation in soils with low redox potential because

excess sulphide is produced (Benoit *et al*. 1999). In more oxidised soils, sulphide is quickly oxidised to sulphate or removed from the pore waters through diffusion, therefore stopping the build-up of this toxic by-product. Also, MeHg reacts with H_2S to produce volatile dimethylmercury (Benoit *et al*. 1999) which can leave the aquatic environment to the atmosphere.

Salinity also affects Hg-DOM binding because other ions, such as chloride, sulphate, and hydroxide, compete with DOM to form metal-ligand complexes, especially in oxic waters. Hg[II] is not present in saline waters as a free Hg^{2+} ion but is complexed with Cl ions depending on the pH and Cl concentrations (see Figure 2.5). $HgCl_2$, a neutral complex, is able to cross the SRB membrane however the formation other Hg-Cl complexes are favoured in high chloride concentrations (Mason *et al.* 1995). Mercury-chloride complexes are thought to be important in high chloride oxic conditions (e.g., seawater) where they form negatively-charged species and inhibit uptake by SRB (Barkay *et al*. 1997, Davis *et al*. 2003); the Hg[II] ion exists primarily as $HgCl_4^{2-}$ and $HgCl_3^-$ (Ullrich *et al.* 2001).

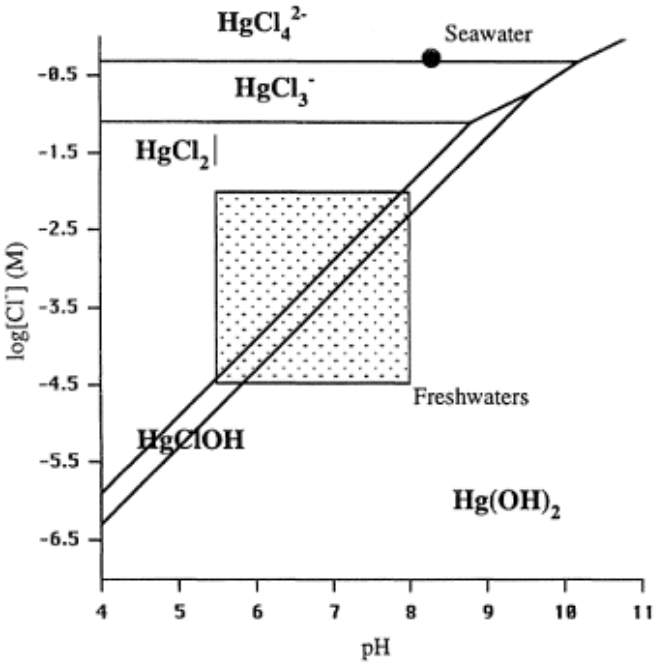

Figure 2.5. Dominance diagram of hydroxo- and chloro-complexes of Hg(II) as a function of pH and chloride concentrations (taken from Morel *et al.* 1998).

Vegetation can also promote MeHg production (Yee *et al*. 2005). Vegetated sediments tend to be more oxidised than unvegetated sediments because plant roots with gas vesicular systems can deliver oxygen to depth (Conrad 1996). Increasing root density will increase the oxic/anoxic area that Hg methylation can take place. Increasing oxygen concentration can also recycle reduced precipitates (i.e. sulphide) in the sediment and stimulate reducing bacteria. Plant roots also exude labile organic carbon in the rhizosphere which stimulates microbial activity and hence methylation (Windham-Myers *et al*. 2009).

It is widely accepted that SRB are the key methylators of Hg[II] at the oxic/anoxic interface in coastal wetlands because there is plentiful supply of sulphate in seawater. However, recent studies have suggested that other pathways of carbon mineralisation are also important for Hg methylation, such as dissimilatory iron reducing bacteria (FeRB) (Morel *et al*. 1998).

2.5.4 pH

Hg bioavailability is also pH-dependent because it controls the partitioning of Hg into the dissolved phase. Low pH generally facilitates the partitioning of metals from sediments and organic matter, suggesting that H^+ competes with Hg[II] for the negatively charged binding sites (i.e., thiol groups) in organic matter (Barkay *et al*. 1997, Ravichandran 2004, Ullrich *et al*. 2001). Furthermore, Hg methylation by methylcobalamin (a vitamin produced by bacteria that provides the 'CH_3' for Hg to be methylated) is favoured at low pH and therefore, MeHg production generally increases at lower pH values (Compeau and Bartha 1984).

2.6 Fate and storage of Hg and MeHg in coastal sediments

Tidal wetlands are retentive environments that trap and store most of the materials they receive, including particulate Hg. Many coastal wetlands store Hg from past anthropogenic activity as well as atmospheric input due to their fine-grained and

organic-rich sediments (Ullrich *et al*. 2001, Spencer 2002). The accumulation of sediment can result in saltmarshes becoming a sink for Hg because it has a high affinity for organic matter.

Total Hg (THg) profiles from coastal wetlands have varied amongst studies, with some reports showing relatively homogenous porewater Hg[II] and sediment phase Hg[II] concentrations with depth, whereas other reports describe variations spatially and with depth. For example, Mitchell and Gilmour (2008) found THg concentrations increased by approximately 20% with depth from 125 ng g^{-1} in surface soils to 153 ng g^{-1} at 12-15 cm depth which reflects previous water quality of the estuary. Hammerschmidt *et al*. (2004) reported constant Hg concentration with depth suggesting either sediment mixing has occurred or a relatively constant Hg input. Table 2.1 shows the range of concentrations of THg in coastal sediments and porewaters.

Table 2.1
Literature references to sediment and porewater THg and MeHg concentrations

Reference	Sediments		Porewater	
	Total Hg (ng g-1)	MeHg (pg g-1)	Total Hg (ng L-1)	MeHg (pg L-1)
Sunderland *et al*. (2006)	10-140	54-1591	-	-
Hung and Chmura (2006)	8-79	-	-	-
Emmerson *et al*. (1997)	110-510	-	-	-
Conaway *et al*. (2003)	20-702	0-2587	-	-
Mitchell and Gilmour (2008)	125-153	100-5000	-	-
Windam-Myer *et al*. 2009	109-559	500-9200	-	-
Choe *et al*. (2004)	58-421	86-14230	2-10	216-4312
Hammerschmidt and Fitzgerald (2004)	43- 345	200-3200	5.6 - 35.9	2000-6100
Hammerschmidt *et al*. (2004)	261-381	862-3881	1-30	2156-8625
Sunderland *et al.* (2004)	8-76	431-1725	10-30	496-1574

MeHg has been shown to have greater spatial, seasonal and vertical variability than THg in sediments (Choe *et al*. 2004). For example, Yee *et al*. (2005) showed spatial variability up to ~10 times between individual samples even though all samples were taken within close proximity to one another. Hg methylation rates are often greatest

within a few centimetres of the surface in saltmarsh sediments, and decrease with depth (Fitzgerald *et al*. 2007, Choe *et al*. 2004). Bioturbation by burrowing invertebrates such as *is* can increase Hg methylation at depth by removing toxic by-products of microbial respiration (e.g. sulphide) as well as providing labile organic substrates to depth. Spatial (and temporal) patterns in factors that control MeHg production (labile carbon, sulphate, pH, and sediment redox potential) will ultimately control the patterns in MeHg concentration (Davis *et al*. 2003, Hammerschmidt *et al*. 2004). MeHg spatial variability can also be controlled by vegetation patterns within the saltmarsh and Yee *et al*. (2005) showed with devegetation experiments that plants promoted sulphate and iron cycling and live root density had one of the highest correlations with Hg methylation.

2.7 Impact of Managed Realignment on Mercury Cycling

Saltmarsh restoration leads to a series of physical changes that may impact on sediment biogeochemistry (Section 2.2) and increase MeHg production, such as a decrease in redox status, an increase in sulphate concentrations and a decrease in pH. A conceptual model showing the redox and sulphur controls on mercury methylation are shown in Figure 2.6.

Prior to restoration, the marsh elevation is low in comparison with the surrounding area. The sediment has higher bulk density and moisture content, and lower organic matter content compared with sediment in natural saltmarshes (French, 2006), resulting in poorly drained sediments. Therefore, MR can create waterlogged and anoxic sediments, which in turn can promote the development of reducing conditions. Fe and Mn cycling can be affected during coastal restoration. A decrease in redox potential can result in Fe and Mn being released into solution. As the sediment becomes reduced, Fe is reduced and released from the Fe oxide-organic complex as a consequence of microbial degradation of organic matter, leaving Hg in solution (Heyes *et al.* 2004, Choe *et al*. 2004). Fe and Mn oxides may only comprise a small fraction of the total sediment composition, however they can be responsible for 10-50 % of the total metal content in the sediment (Zhong and Wang 2008).

Introducing sulphate to the sediments through tidal inundation, as well as reducing conditions, can stimulate SRB and increase MeHg concentrations. In addition pH may also decrease following de-embankment and tidal inundation (Blackwell *et al*. (2004)) and these changes can increase the partitioning, mobility and bioavailability of Hg (Macleod *et al*. 1999)**.**

Previous research has indicated tidal wetlands are efficient areas for microbial MeHg production (Marvin-DiPasquale and Agee 2003). A primary source of MeHg production is within the sediment through biotic mechanisms. Therefore, increasing wetland area through restoration has the potential to produce hotspots for MeHg production (Marvin-DiPasquale and Cox 2007).

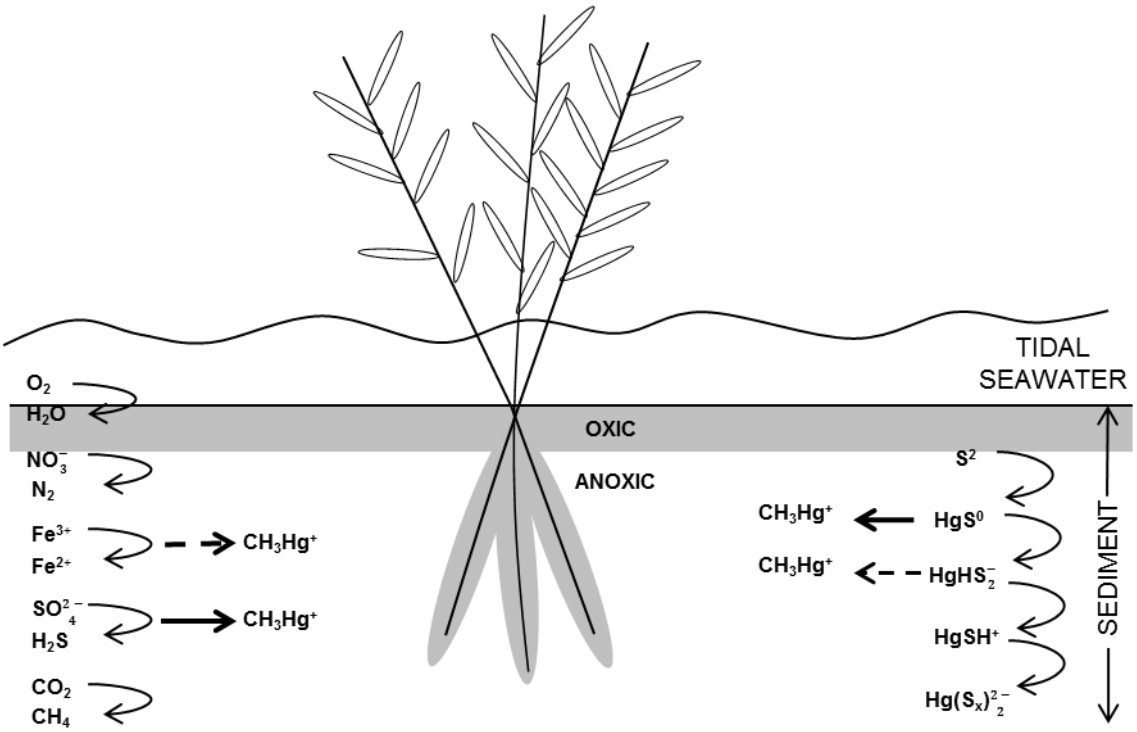

Figure 2.6. Conceptual model of the vertical distribution of redox reactions that influence the production of MeHg in submerged coastal sediments and of the reoxidation of reduced inorganic electron acceptors by O_2 in the oxic layers at the sediment-water interface and the rhizosphere of aquatic plants (adapted from Conrad 1996).

2.8 Research Gaps

2.8.1 Aim and Research Objectives

There have been numerous studies on the ecological development in restored coastal wetlands (Wolters *et al*. 2008, Wolters *et al*. 2005, Hughes *et al*. 2009, Garbutt *et al*. 2006), however, few studies have focused on geochemical changes that occur following inundation (Emmerson *et al*. 2000, Macleod *et al*. 1999, Andrews *et al*. 2006, Kolditz *et al*. 2009, Blackwell *et al*. 2004, Spencer *et al*. 2008) especially focusing on Hg speciation and bioavailability (Yee *et al*. 2005). Mercury cycling in managed realignment sites is poorly understood, indicated by an almost complete lack of literature on the subject. By the mid-2090s, global sea level is predicted to reach 0.22 to 0.44 m above 1990 levels, and is rising at approximately 4 mm yr^{-1} (Bindoff *et al*. 2007). MR is a technique being increasingly implemented across Europe and the USA to deal with sea level rise because it is an economically viable option compared to improving and repairing current sea defences. It also creates habitats and fulfils the UK governments' commitment to the European Union Habitats Directive (92/43/EEC) which specifies a no-net-loss policy. Managed realignment restores a tidal connection. A return to tidal inundation and anaerobic conditions may convert inorganic Hg to MeHg. Previous research has indicated tidal wetlands are efficient areas for microbial MeHg production. Therefore, increasing wetland area has the potential to produce hotspots for MeHg production (Marvin-DiPasquale and Cox 2007). Whilst coastal restoration creates habitats and promotes wildlife development, it may create an indirect link for methylmercury to enter the food chain. The need to understand the controls on Hg mobility and partitioning within these newly created coastal wetlands is becoming increasingly pressing due to projected global sea level rise and the increasing use of this technique.

The overall aim of this PhD thesis is to understand the controls upon Hg dynamics in coastal sites, with specific emphasis on the effects of ecosystem restoration on MeHg production. The following aims and objectives have been developed to form three results chapters outlined below.

2.8.1.1 Chapter 4

Given the lack of information on Hg cycling in managed realignment sites the first focus of research is to provide a baseline data set of Hg concentrations in restored coastal wetlands with the aim to give details on Hg spatial variability, the association between Hg concentration and indicators of saltmarsh development, and finally to explore how Hg speciation changes with time since de-embankment.

Aim: To provide baseline data on Hg biogeochemistry in restored coastal wetlands.

Objective 1: To assess the spatial variability of THg and MeHg concentrations in natural and de-embanked saltmarsh sediment over a range of spatial scales.

It is hypothesised that spatial variance in THg and MeHg concentrations are greater in natural saltmarshes than in restored wetlands, because natural saltmarshes show greater spatial heterogeneity in controlling factors such as vegetation, topography and physico-chemical parameters.

Objective 2: To access the vertical distribution of THg and MeHg concentrations in natural and restored saltmarsh sediment.

It is hypothesised that THg in natural saltmarshes will reflect historical Hg deposition patterns, whereas the restored sediment profiles will contain highest concentrations in the surface sediments reflecting recent sediment deposition. It is hypothesised the MeHg profiles in the natural saltmarsh will be highest at the anoxic/oxic boundary within the top 10 cm of the sediment profile. The peak concentration in the restored sediment is hypothesised to be greatest in the surface sediments because these sediments contain more reducing environments.

Objective 3: To examine the association between Hg speciation and indicators of saltmarsh development.

It is hypothesised that THg concentrations will increase with % LOI because of the association between Hg and organic matter; and the proportion of MeHg as THg (% MeHg) will increase with moisture content because increased moisture content is indicative of anoxic sediments, conditions known to promote Hg methylation.

Objective 4: To explore how changes in these physico-chemical conditions and Hg biogeochemistry have changed with time since de-embankment and hence ecosystem development.

It is hypothesised that recently-restored sites will have higher bulk density and lower LOI and moisture content than natural sites, but these properties will become more similar to those in natural sites with increasing time since de-embankment. It is expected that MeHg concentrations will be highest in recently-restored sites and decrease with time since de-embankment, as the sites drain and become less waterlogged and anoxic.

2.8.1.2 Chapter 5

Rewetting sediments with saline water can promote MeHg production (Gilmour *et al*. 2004, Kelly *et al*. 1997). Firstly, high decomposition activity stimulated from fresh water vegetation dying and providing a large amount of labile organic carbon, can promote an anaerobic environment that will further stimulate SRB activity. Secondly, initially sulphide levels do not reach inhibitory levels to Hg methylation by SRB and so high levels of MeHg can persist for significant periods until a reducing environment is established. Also, sulphide could be oxidised at low tide, preventing the build-up of inhibitory levels for Hg methylation. Finally, flooding of soils has been shown to create acidic porewater (Blackwell *et al*. 2004). Metal availability is more pronounced in sediment with a lower pH value because the metal partitions into the aqueous phase which can be more easily absorbed by SRB. However, the initial impact of coastal restoration on Hg methylation is unknown and could generate large MeHg releases with potential negative effects on fish and other wildlife.

Aim: To examine the short-term effects of flooding on methylmercury concentrations in agricultural soil.

Objective 5: To examine the net MeHg concentrations in terrestrial soils incubated under different combinations of *Eh* (oxic, anoxic and fluctuating) with water containing sulphate concentrations comparable to seawater and water containing no sulphate, over a period of eight weeks.

It is hypothesised that MeHg production occurs under anoxic or fluctuating oxic-anoxic conditions, but not under permanently oxic conditions; MeHg production is higher under fluctuating oxic-anoxic conditions than under permanently anoxic conditions; and under suitable Eh conditions, MeHg production is higher in soils incubated in saline water than in DI water.

2.8.1.3 Chapter 6

MeHg production is dependent on many environmental factors and is governed by habitat specific processes. Sediment THg concentration does not necessarily control MeHg concentrations, and in fact THg concentrations have often been found to be poorly correlated with concentrations of MeHg in sediment and porewater (Heim *et al*. 2007). Inorganic Hg[II] is most susceptible to methylation when it is in the dissolved state (Davis *et al*. 2003). Therefore, dissolved-solid phase partitioning reactions are important for regulating Hg bioavailability (Krabbenhoft *et al*. 2005). It is important to understand the controls on Hg methylation in the surface sediments in restored coastal wetlands. Surface sediments in the managed realignment sites are typically more waterlogged, anaerobic and less cohesive than in natural saltmarshes creating potentially key Hg methylating conditions. Furthermore, the sediment becomes anoxic within millimetres of the surface allowing Hg[II] bound to the sediment to be more easily partitioned into the aqueous phase, a prerequisite for Hg to be methylated.

Aim: To understand the controls on mercury biogeochemical cycling in restored coastal wetlands, with specific emphasis on the effects of ecosystem restoration on Hg methylation.

Objective 6: To explore the association between Hg methylation and environmental parameters in restored coastal wetlands

Objective 7: To explore how controls on Hg methylation change with time since de-embankment and hence ecosystem development.

It is hypothesised that sulphide concentration will inhibit Hg methylation in newly de-embanked sites that are waterlogged and highly anoxic. In older sites that more closely resemble natural saltmarsh sediments, it is hypothesised that the sediments will be too oxic for sulphate reduction and so Hg methylation will be primarily produced by iron reducing bacteria.

Chapter 3: Methodology

3.1 Introduction

This chapter outlines the methodology used in order to answer the objectives set out in Chapter 2, Section 2.8. First, a detailed site description is given with a rationale as to why these sites were selected. Second, the sampling design is presented including the methods of sampling and sample storage. Finally, the laboratory analyses used to quantify sediment and porewater properties are detailed including sample preparation, quality control and justification as to why certain methods have been used. Statistical analyses used to test each hypothesis are described in detail in their respective results chapters (Chapter 4, 5, and 6).

3.2 Site Description

Field sampling was conducted in October 2012 at Orplands Farm, Ferry Lane, and Northey Island, on the southeast coast of England (Figure 3.1; Table 3.1). Photographs of the sites are shown in Figure 3.2. Orplands Farm (on the Blackwater Estuary) was artificially de-embanked through managed realignment in 1995, whereas Ferry Lane (Colne Estuary) and Northey Island (Blackwater Estuary) were inadvertently de-embanked in 1945 and 1897 respectively, during storm surges resulting in tidal inundation (Mossman *et al.* 2012). These three restored sites are therefore on a temporal gradient of 17 (Orplands), 67 (Ferry Lane) and 115 (Northey Island) years since inundation. All three sites were previously used for agriculture, although practices would have differed significantly given the differences in age. Mean spring tidal range for the estuary is 4.7 m (Garbutt *et al*. 2006). They all have a similar underlying geology (Thames Group) containing silty clay/mudstone, sandy silts and sandy clayey silts of marine origin (British Geological Society, 2016).

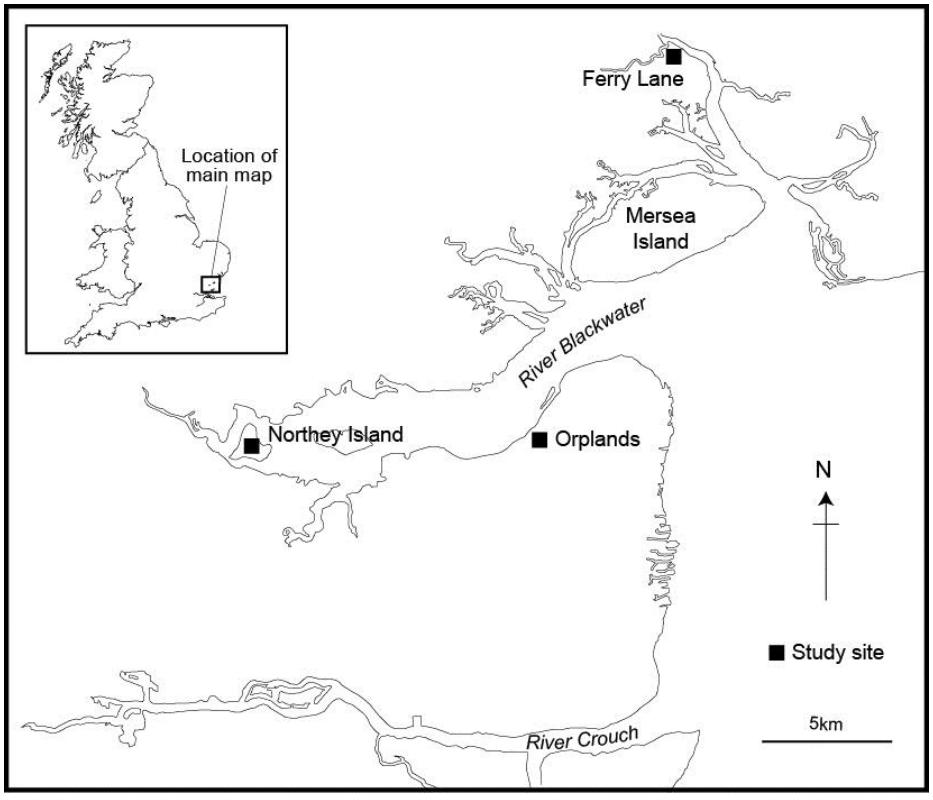

Figure 3.1. Map of the study area showing sampling sites at Orplands, Ferry Lane, and Northey Island.

Table 3.1
Site locations and details

Site	Estuary	Latitude/Longitude	Site Area (ha)	Time since breach (years)
Orplands	Blackwater	51º43.28N, 0º52.20E	35	17
Ferry Lane	Colne	51º51.15N, 0º57.46E	6	67
Northey	Blackwater	51º43.27N, 0º43.20E	78	115

a)

b)

Figure 3.2. Photograph's of a) Orplands managed realignment site and b) Northey Island natural saltmarsh.

3.2.1 Sampling design and locations

a) Objectives 1, 2, 3 and 4 (Chapter 4)

In order to answer Objectives 1, 3 and 4 sediment concentrations of THg and MeHg, as well as physical sediment properties indicative of saltmarsh development, were examined at three spatial scales (small scale, 1 m; intermediate, c. 15-50 m; and large scale, c. 15 km) across three paired de-embankment sites and adjacent natural saltmarshes in southeast England (Figure 3.1). Areas of natural saltmarsh occur adjacent to all three restored wetlands. Both restored and natural wetlands contain typical saltmarsh vegetation, although species richness, composition and structure differ (see Garbutt and Wolters 2008 for full vegetation description).

De-embanked and adjacent natural saltmarshes were sampled (six wetlands in total) in October 2012, with the natural saltmarshes serving as a control for between-site differences unrelated to time since breach. Samples were collected from vegetated saltmarsh in a hierarchical design in order to identify the spatial scale(s) accounting for most of the variability in THg and MeHg concentrations and physico-chemical properties. The three sites were located approximately 15 km apart and on two different estuaries, allowing examination of within-region variability as well as that associated with time since de-embankment of the restored wetlands. For each wetland, ten random sampling coordinates were generated between 15-50 m apart to examine intermediate (within-site) variability, and field sampling was conducted as close to these locations as possible. Each location was sampled in triplicate within one square meter, in order to quantify smaller-scale variation in Hg concentrations and physico-chemical conditions.

Sediment cores were collected using acid-cleaned, clear polycarbonate tubes (30 cm long and 7.5 cm diameter), bevelled at one end to aid insertion. Cores were capped immediately at both ends and kept upright and chilled in a cooler during transport back to the laboratory. Once in the laboratory, the sediment cores were frozen and stored at -20°C until analysis. Previous research has indicated significant differences in vegetation and sediment properties between restored and natural sites (Garbutt and

Wolters 2008, Wolters *et al.* 2005, Windham-Myers *et al*. 2009), and so the depth and breadth of the methylation zone is also likely to differ. Analysing each core at multiple depths would have generated an enormous number of samples and compromised the examination of spatial variability. Therefore, sediment was composited along the entire length of each core to ensure the zone of methylation was captured in individual samples while keeping the total number of samples manageable**.**

Sediment cores were removed from the polycarbonate tubes in an anaerobic glove box. A sub-sample, the full length of the core, was taken from the centre of the core, lyophilised and homogenised for THg and MeHg analysis. The remainder of the core was used for determination of LOI, moisture content and bulk density. Data reported are composites for the entire core.

In order to answer Objective 2, six paired cores were taken from Orplands site (three cores from the managed realignment site and three cores from the natural saltmarsh). Orplands was chosen because this site was most recently restored and so differences between the restored and natural sites are likely to be greatest. All three cores were taken within 1 m^2 and the location from the managed site was at the same elevation as the location within the natural saltmarsh. This was an attempt to control for differences in the hydrological regime at the sites. Therefore, the location of the sampling site in the restored site was at the landward edge of the site, whereas for the natural site the cores were collected from the middle of the site. These cores were subsampled every 2 cm until 26 cm depth for THg and MeHg analysis.

b) Objectives 5 (Chapter 5)

In order to answer Objective 5, a sediment plug method was used to expose the surface of a sediment plug of fixed thickness to different treatments (for full details see Allert and Mackin 1989; Figure 3.3). Soil was collected from a bare agricultural field adjacent to Orplands managed realignment site from a depth of 0-5 cm. This site was chosen because sediment data from Orplands suggested low localised Hg contamination sources and the soils are representative of those flooded in south-east

England where de-embankment is practiced. The soil was sieved (mesh size 2 mm) and chilled at 4 °C until needed. Prior to analysis, samples were thawed and sub-samples were placed into acid washed clear polycarbonate tubes (2 cm deep, 5 cm diameter). Three soil sub-samples were analysed to allow the initial THg and MeHg concentrations to be determined as well as the relative standard deviation (RSD).

Plugs were exposed to two treatments in a full-factorial design: (i) with two levels: saline vs deionised (DI) water (control) and (ii) with three levels: constantly anoxic vs fluctuating (oxic/anoxic) vs constantly oxic (control). Using a factorial combination, the experiment had six combinations (Oxic/DI, Anoxic/DI, Fluctuating/DI, Oxic/Saline, Anoxic/Saline, and Fluctuating/Saline). *Eh* treatments used in this experiment were chosen to be representative of field conditions in restored sites. The plugs were placed in sealed acid washed glass tanks and flooded with 30 L of either DI water or a commercial sea salt mix (Sigma-Aldrich sea salts) which contained all salts, including sulphate, in amounts representative of levels found in natural seawater (salinity of 35 ppt). The overlying water was then continuously purged with nitrogen (anoxic) or oxygen (oxic) and a stirrer ensured a well-mixed water reservoir (Figure 5.1). The fluctuating oxic/anoxic treatment was alternated between oxygen and nitrogen purging every 24 hours (alternating every 12 hours to mimic tidal cycles was not feasible due to restrictions on laboratory access). The experiment was run for eight weeks. A total of 24 sediment plugs were used, allowing duplicate sediment plug samples to be removed daily during week one, weekly for the following three weeks, and then fortnightly for the remainder of the experiment. Three soil sub-samples were analysed to allow the initial THg and MeHg concentrations to be determined. The relative standard deviation was calculated because it is a useful measure for comparing the uncertainty between different measurements.

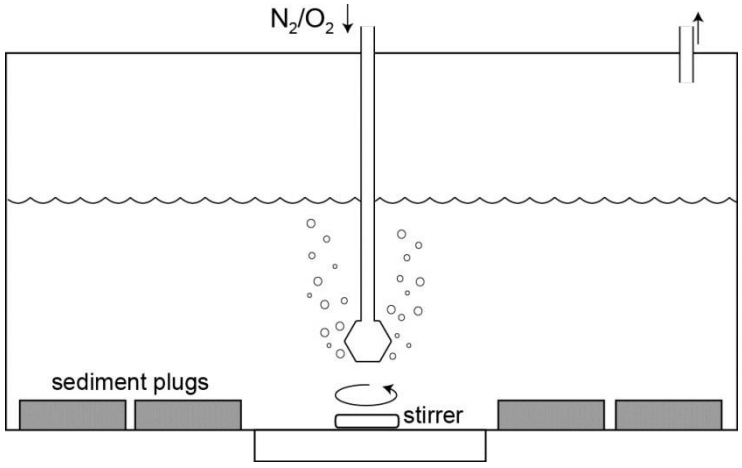

Figure 3.3. Diagram of sediment plug experiment with multiple samples each with one exposed surface.

As discussion in Section 2.5.1, DOC is known to be an important control on Hg methylation. In order to remove any confounding influences relating to DOC, de-ionised water with zero DOC content was used in all treatment combinations. Doing so, afforded a high degree of experimental control, ensuring that all observed effects could be attributed to factors other than DOC (i.e. salinity or redox).

The only advantage of changing DOC content as an experimental factor would be to examine the potential interactive effects with salinity and redox. Although doing so was beyond the scope of the research questions for this thesis and was not possible within the time and budgetary constraints.

c) Objectives 6 and 7 (Chapter 6)

In order to answer Objectives 6 and 7 surface sediment samples (upper 2 cm) were collected using acid-cleaned airtight Nalgene centrifuge bottles. Sediment was collected from the upper 2 cm of the substrate because this is the sediment depth which typically has the highest MeHg production and has maximum interaction and flux with the overlying water column during tidal inundation (Grenier *et al*. 2010). Samples were collected in February and March 2013 using the same sampling design as that used for Objective 1, 3 and 4. The bottles were filled with sediment, leaving no head space in an attempt to minimise oxidation of reduced species. Samples were

chilled in a cooler during transport back to the laboratory and stored at -20°C until analysis. The solid phase was separated from the liquid phase via centrifugation for 20 minutes at 3,000 revolutions per minute (rpm). In a N_2 flushed glove box, supernatant from the triplicate samples taken at each location were composited to ensure adequate sample volume for Hg analysis. The composited supernatant was subsampled for THg, MeHg, Cl^-, SO_4^{2-}, Fe, Mn, Fe^{2+} and S^{2-} analysis. The solid-phase material from triplicate samples was scraped out of the bottom of the centrifuge bottles, composited and transferred into an acid-cleaned airtight Nalgene bottles ready for the following analyses: THg, MeHg, pH, LOI, acid volatile sulphur (AVS), chromium reducible sulphur (CRS), as well as a suit of trace metals including aluminium (Al), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni) and potassium (K).

All equipment used for subsampling and sample analysis was rigorously cleaned with 10 % nitric acid and rinsed with deionised water. All chemical reagents were trace-metal or ACS grade.

3.3. Sediment Sample Analysis

Sediment samples were collected in sample bags and frozen at -20 °C until analysis. Freezing samples is the recommended and widely used method for sample storage for Hg analysis (Hall *et al*. 2009; Bloom *et al*. 2004; Hammerschmidt and Fitzgerald, 2001; Bloom et al. 1997). MeHg concentration is strongly mediated by microbial communities and therefore freezing is the recommended storage option to preserve MeHg concentrations in sediments. It has been noted that freezing can have a small impact on the inorganic speciation of THg in the aqueous phase (Parker and Bloom, 2005) however the impact on MeHg would be greater and therefore is the preferred method.

Recoveries of some reference material for THg and MeHg were greater than 100 %. Recoveries greater than 100 % are not unusual for Hg analysis, especially for MeHg. Firstly, artefactual MeHg formation is well documented during MeHg extraction and analysis (Hintelmann, 1999) from the conversion of THg to MeHg. Distillation, the extraction method used here, is the method preferred to limit artefactual MeHg however it is likely a small amount of THg was converted to MeHg during the extraction process.

Secondly, the calibration curve will have had a different chemistry than the certified reference material digestion/extraction. Samples are treated with chemicals that would eliminate the interferences, but could enhance the response. It is imperative that the chemistry of the calibration standards is as close as possible with that of the samples. This is the case of ascorbic acid addition, added to eliminate the halides interference in the distillation extracts. Although all efforts were made to matrix match, some differences would have occurred. The calibration curve was made to match the samples as closely as possible, however the chemistry of the method used for Hg quantification of the standard reference material is unknown and therefore probably differed to that used for sample analysis.

3.3.1 Total mercury

Mercury analyses were performed at the Biotron Centre for Climate Change Research Analytical Services Laboratory at the University of Western Ontario, London, Canada. Total Hg was analysed using a Milestone® Direct Mercury Analyser-80 (USEPA 1998). A direct solid-sample analysis was possible using a direct Hg analyser (Han *et al*. 2003, Roy and Bose 2008). The lyophilised sediment sample was thermally decomposed and the combustion products are carried through to a hot catalyst bed. The Hg vapours are trapped on a gold amalgamator and subsequently desorbed and determined using atomic absorption spectrophotometry (AAS) at 254 nm. Direct solid-sample analysis is extremely efficient, accurate and produces reproducible results. Blanks, sample duplicates, and standard reference material (MESS-3, a marine sediment reference material for trace metals certified by National Research Council Canada, certified at 0.091± 0.009 mg Hg/kg) were analysed every 10 samples. Duplicate measurements were conducted and precision (relative standard deviation; % RSD) was 5 % (*n* = 20). The average blanks were below detection. The mean recovery of THg in MESS-3 was 109 % (100 – 121%, n = 25). The method detection limit was 0.18 ng Hg.

3.3.2 Methylmercury

Sediment MeHg was extracted using alkaline (potassium hydroxide and methanol) extraction (Ogorek and Dewild 2013) and measured by CV-AFS (Bloom 1989) using a Tekran 2700 (USEPA 2002). The alkaline digestion method has significant advantages over other techniques (e.g. distillation) especially when samples contained high Hg[II] concentrations (Liang *et al*. 1996). Accuracy was measured by measuring method blanks, a sediment reference material (ERM-CC580, an estuarine sediment certified at 0.075 ± 0.004 mg MeHg/kg), and spiked samples. The mean recovery of MeHg in ERM-CC580 was 88 % (71 – 109 %, n = 33). Spike recoveries averaged 109 % (74 – 123 %, n = 23). Precision (% RSD) of MeHg averaged 6.9 % (n = 13). The analytical method detection limit was 0.0054 ng/L.

3.3.3 pH

The pH was determined using a HANNA pH meter, calibrated with two calibration solutions at pH 4 and 7. The pH was determined in the laboratory within 24 hours of the sample defrosting by weighing 20 g of sediment into a beaker and adding 40 mL of deionised water. The slurry was stirred vigorously and allowed to stand for 30 min prior to analysis (Radojević and Bashkin 1999). The pH electrode was rinsed with deionised water and dried between sample readings.

3.3.4 Bulk density, moisture content and loss on ignition

Sediment bulk density and moisture content were measured for each sample by weighing a known volume of sample before and after drying for 24 hours at 105 °C. Bulk density was calculated by applying the following formula:

$$\text{Bulk density (g cm}^{-3}\text{)} = \text{dry weight (g) / volume (cm}^{3}\text{)}$$

Sediment loss on ignition (LOI) is used as an approximation of organic matter and was estimated by the mass lost upon ignition at 450 °C for four hours. Loss on ignition is the most commonly used method to approximate organic matter content (Marvin-DiPasquale and Agee 2003, Windham-Myers *et al*. 2009, Heim *et al*. 2007). It is a rapid and inexpensive method. Recent literature indicates that good repeatability can be achieved as long as the method is carefully controlled (e.g., Heiri *et al.* 2001). Loss on ignition can be used only as an approximation of organic matter content because other components of the soil can be lost upon ignition, leading to over-estimation of organic matter content, e.g. dehydration of clay minerals or metal oxides, loss of volatile salts, or loss of inorganic carbon in minerals (Rowell 2014, Heiri *et al.* 2001).

The % RSD for triplicate samples analysed for LOI averaged 2.8 %. It was not possible to analyse triplicate samples for bulk density or moisture content because the entire core was used during initial calculations. However, triplicate cores were taken within 1 m^2 and so these data were used to check method precision as well as the small-scale

variability. The % RSD of triplicate samples taken within 1 m^2 of each other is given in Table 3.2.

Table 3.2

Percent relative standard deviation (% RSD) of triplicate samples taken within 1m of eachother

Location	Total Hg	MeHg	LOI	Moisture Content	Bulk Density
Orplands					
Restored	8.3	54.5	5.9	3.2	7.6
Natural	7.8	24.7	5.9	2.0	6.8
Ferry Lane					
Restored	9.8	14.2	6.7	1.6	2.8
Natural	13.4	17.3	8.4	2.7	8.7
Northey					
Restored	6.6	38.7	7.9	2.6	7.8
Natural	10.5	20.7	6.2	1.5	5.8

3.3.5 Geochemical analysis

Sediment samples were analysed for trace elements using an aqua regia hot-plate extraction (3:1 HCl:HNO_3) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The ICP-OES instrument used was a Vista-PRO^{TM} spectrometer with a SPS3 autosampler. The ICP-OES was calibrated at the beginning of each run with standards made from a multi-element standard solution covering the range of sample concentrations analysed. To overcome interference effects due to plasma suppression from easily ionisable elements, the standards were matrix matched to the composition of the samples as best as possible. A calibration standard was incorporated after every ten samples to monitor drift in the analyte signal over time. Each batch of 10 samples was accompanied with one certified reference material (LGC-6137, aqua regia digested marine estuarine sediment, see Table 3.3), one analytical duplicate and one method blank. Limits of detection were calculated as three standard deviations of the mean of ten replicate readings of a blank sample.

Table 3.3

Trace metals in reference material LGC-6137

Trace metals	Levels (mg kg^{-1})	Recovery (%)	Min-Max (%)
Cu	31.6	97.4	90.8 - 104.5
Fe	30700	99	82.7 - 107.7
Mn	665	99.6	93.2 - 104.6
Ni	31.5	96.7	91.0 - 109.9
Pb	73.0	93.3	83.0 - 102.8
Zn	231	101.9	96.2 - 109.4

3.3.6 Total carbon and nitrogen

Lyophilised sediment (5-10 mg) was weighed into ultra clean tin caps and weighed on a microbalance to a precision of 1 μg. The samples were analysed for total carbon and nitrogen using an elemental analyser (Flash elemental analyser, series 1112, Thermo-Finnigan, Bremen, Germany). Each batch of 10 samples was accompanied with one analytical duplicate and one method blank.

3.3.7 AVS and CRS

The oxidation of organic matter by sulphate reducing bacteria (SRB) produces H_2S which reacts with abundant Fe to form Fe sulphides (Lasorsa and Casas 1996). Sulphide actively scavenges trace metals to form insoluble metal sulphides in anoxic environments and reduces their availability to organisms (De Lange *et al.* 2008). Sulphide can also react with other divalent transitional metals (e.g. Hg[II]) to form insoluble precipitates and therefore in marine sediments the metal concentration in porewater is largely controlled by sulphide concentration (Allen *et al.* 1993). Acid volatile sulphide (AVS) is an operational definition for the evolution of H_2S gas that is derived from adding HCl to a sample (Rickard and Morse 2005). AVS is metastable and measures both dissolved sulphur species (S^2) and HCl-reactive sulphide minerals including mackinawite (FeS) and greigite (Fe_3S_4). AVS transforms on aging to pyrite (FeS_2), a thermodynamically stable sulphide and is the major reservoir of reduced sulphur in sediments (Lasorsa and Casas 1996). Methods for the determination of AVS and pyrite are operationally defined and vary between studies (Billon *et al.* 2001a,

Ubuka *et al*. 2001, Canfield *et al*. 1986, Allen and Parkes 1995). It is important to clearly state the method used and take this into consideration when interpreting results.

Reduced sulphur species in surface sediments were determined after the conversion to H_2S gas following a sequential extraction method, as previously described (Canfield *et al*. 1986, Billon *et al. 2*001b). Briefly, AVS was assayed by adding 20 mL of 6 M deoxygenate HCl and 100 mL deoxygenated deionized water to 1 g of wet sediment, and trapping the H_2S generated in NaOH traps (personal communication with G. Billion). The concentration of S^{2-} in the NaOH traps was determined by titration with 0.01 M lead perchlorate and a sulphide-selective electrode (personal communication with Marvin-DiPasquale (USGS)).

After AVS, the resulting solution was treated with chromium[II] chloride to extract the total reducible inorganic sulphide (TRIS), consisting of sulphides not extracted by the AVS digestion, for example pyrite and elemental sulphur (Billon *et al.* 2001a). Cr[II] was made by passing $CrCl_3$ through a Jones' column (granulated zinc covered with 2 % mercury[II] Cl solution) and then acidified to 0.5 M with HCl. While continuously flushing the sample with N_2, 40 mL 1 M $CrCl_3$ and 20 mL of deoxygenated 6 M HCl were added. The sample was then heated to 80-100 °C for 1 hour. H_2S generated is trapped in NaOH traps and assayed by titration with 0.01 M lead perchlorate and a sulphide-selective electrode. The method detection limit was 5 ng g^{-1}.

3.4 Porewater Sample Analysis

Samples were collected in centrifuge bottles, leaving no head space in an attempt to minimise oxidation of reduced species. Samples were chilled in a cooler during transport back to the laboratory and stored at -20°C. Freezing samples is the recommended and widely used method for sample storage for Hg analysis (Hall *et al*. 2009; Bloom *et al*. 2004; Hammerschmidt and Fitzgerald, 2001; Bloom et al. 1997). MeHg concentration is strongly mediated by microbial communities and therefore freezing is the recommended storage option to preserve MeHg concentrations in

sediments. It has been noted that freezing can have a small impact on the inorganic speciation of THg in the aqueous phase (Parker and Bloom, 2005) however the impact on MeHg would be greater and therefore is the preferred method. Once thawed, the sediment was centrifuged for 20 minutes at 3,000 rpm. The porewater supernatant was then filtered through a 0.45 µm PTFE filter under anaerobic conditions.

3.4.1 Total Mercury

Mercury porewater concentrations were determined according to USEPA method 1631, Revision E (USEPA 2002). This method is based on tin [II] chloride reduction of Hg[II] to Hg[0] vapour, trapping Hg[0] on gold plated sand followed by thermal desorption and detection via CV-AFS. Each analytical batch of 10 samples was accompanied by the analysis of quality assurance samples including one certified reference material (1ppm, from Sigma Aldrich), one matrix spiked sample, one analytical duplicate and one method blank. The limit of detection was 0.27 ng/L.

3.4.2 Methylmercury

The sample was heated and purged with nitrogen, distilling the water and MeHg into a collection vial. This process eliminates matrix interferences such as organic matter, particulates, and sulphides that would otherwise hinder the subsequent steps (a limitation for other separation techniques). The addition of sodium tetraethylborate ($NaBEt_4$) to the solution ethylates the methylmercury, making it volatile and easily purged from solution. The volatile MeHg is then trapped and pre-concentrated, before going through the CV-AFS detector. $NaBEt_4$ has many advantages over other separation methods because it easily isolates MeHg from the sample matrix and further clean-up steps are not needed. Each analytical batch of 10 samples was accompanied by the analysis of quality assurance samples including one certified reference material (1ppm, from Sigma Aldrich), one matrix spiked sample, one analytical duplicate and one method blank. The analytical method detection limit was 0.0054 ng/L.

3.4.3 Anions: Sulphate and Chloride

Chloride (Cl^-) and sulphate (SO_4) were analysed by ion exchange chromatography (ICS-2500, Dionex). Anions were separated on an IonPac AS18 analytical column with an IonPac AS18 guard column. The eluent used was 100 mM KOH pumped by a GP50 gradient pump at a flow rate of 0.25 mL min^{-1}. Calibration standards covering the range of sample concentrations analysed were made using sodium sulphate and sodium chloride. One standard, a duplicate sample and a method blank were analysed throughout each analytical run in order to assess accuracy and precision.

3.4.4 Reduced Iron (Fe^{2+})

Porewaters were immediately stabilised with a buffered phenanthroline solution (1 mL sample:4 mL buffered phenanthroline) which forms an intense orange coloured Fe(o-phen)$_3^{2+}$ complex that can be detected using a UV spectrometer. The intensity of the orange solution is directly proportional to the amount of Fe^{2+} present. The absorbance maximum for the ferrous-phenanthroline ion is at 510 nm. Samples were stored in the dark until analysis. One sample blank and one standard sample was analysed every ten samples to check for contamination and drift.

3.4.5 Geochemical analysis (Fe and Mn)

Porewaters were acidified to 1 % with nitric acid and analysed on an ICP-OES (see Methods, Section 3.3.5 for further details).

3.4.6 Sulphide

Porewaters were immediately stabilised with a sulphide antioxidant buffer, SAOB (5 mL sample:5 mL SAOB), and analysed within 24 hours. The sample was titrated against 0.01 M lead perchlorate using a sulphide-selective electrode (Baumann 1974, Billon *et al.* 2001a, Marvin-DiPasquale (USGS) personal communication). The method detection limit was 500 µg L^{-1}.

Chapter 4: Temporal and spatial distributions of sediment mercury in restored coastal saltmarshes

The spatial distribution data from this chapter have been published in Marine Chemistry (Morris *et al*. 2014) see Appendix 1.

4.1 Introduction

Coastal wetlands can show high rates of methylmercury production (Krabbenhoft *et al*. 1999, Lacerda and Fitzgerald 2001, Heim *et al*. 2007, Hall *et al*. 2008, Mitchell and Gilmour 2008). Microbial Hg methylation could be promoted in restoration sites following de-embankment for the following reasons; 1) SRB thrive in anoxic sediments, 2) there will be an expansion of the root:soil interface (rhizosphere) and 3) fluctuations in redox conditions caused by wetting and drying during tidal cycles will cause the re-oxidation of sulphide and production of sulphate and increase carbon availability. Therefore, restoration sites could produce Hg methylation 'hotspots' (French 2006, Hall *et al*. 2008). In contrast, sulphide, an end-product from sulphate reduction, can severely inhibit Hg methylation (Compeau and Bartha 1984, Benoit *et al*. 1999) at concentrations higher than ~30 µM (Craig and Moreton 1983, Morel *et al*. 1998, Benoit *et al*. 2001) and in poorly drained sediments, such as those found in the early stages of de-embankment, sulphide may limit Hg methylation. Therefore, the implications of restoration on Hg methylation are uncertain. In addition, the zone of Hg methylation can vary by an order of magnitude depending on specific site characteristics (Bloom *et al*. 1999, Choe *et al*. 2004) and in these sites where physico-chemical sediment characteristics may vary significantly with depth, rates, patterns and controls on Hg methylation are unclear.

The following chapter outlines the results and discussion addressing the four objectives set out in Chapter 2, Section 2.8.1.1.

Aim: To provide baseline data on Hg biogeochemistry in restored coastal wetlands.

Objective 1: To assess the spatial variability of THg and MeHg concentrations in natural and de-embanked saltmarsh sediment over a range of spatial scales.

Objective 2: To access the vertical distribution of THg and MeHg concentrations in natural and restored saltmarsh sediment.

Objective 3: To examine the association between Hg speciation and indicators of saltmarsh development.

Objective 4: To explore how changes in these physico-chemical conditions and Hg biogeochemistry have changed with time since de-embankment and hence ecosystem development.

4.1.1 Sampling Strategy

For a full sampling strategy see Methodology, Section 3.2.1. The sediment samples were analysed for THg, MeHg, LOI, bulk density and moisture content. A summary of the analytical methods used are shown in Table 4.1.

Table 4.1
Summary of methodology for Chapter 4

Sample	Number of samples	Replicates	Subsampled?	Total number of samples
Sediment cores	60	3	Composited	180
	2	3	Every 2cm until 26 cm depth	78

Analyte of interest	Method of quantification	Full method description
Sediment THg	AAS	Section 3.3.1
Sediment MeHg	CV-AFS	Section 3.3.2
LOI	Ignition at 450 °C	Section 3.3.4
Bulk Density	Calculated	Section 3.3.4
Moisture Content	Calculated	Section 3.3.4

4.1.2. Statistical Analyses

Statistical analyses were performed using SPSS 19.0 and R 3.1.1 (R Development Core Team 2013) for Windows. Linear mixed-effect models were used to test for effects of site type (restored, natural) and the interaction between type and site (Orplands, Ferry Lane, Northey Island) on THg and MeHg sediment concentrations, as well as on physico-chemical variables indicative of saltmarsh development (LOI, moisture content, bulk density). In addition, the relative contribution of different spatial scales (site, location, replicate) to the total variation in these response variables was calculated for each site type separately (R packages "lme4" and "HLMdiag"; Bates *et al*. (2014), Loy and Hofmann (2014)). Where necessary, variables were transformed to ensure normality ($\log_{10}$(THg), sqrt(MeHg), arcsin(sqrt(LOI)), arcsin(sqrt(moisture content)), sqrt(bulk density)). Spearman's rank correlation analyses were used to test the association between THg and MeHg concentrations and physico-chemical variables. Spearman's rank correlation was selected as it assumes monotonic relationships, including non-linear ones. Temporal changes following de-embankment were assessed by comparing THg, MeHg and physico-chemical variables across sites. Differences between the mean values for paired sites (restored minus natural) were calculated to account for between-site differences unrelated to time since de-embankment. Errors were propagated to give 83% confidence intervals, the overlap of which allows differences with time since de-embankment to be assessed. Payton *et al*. (2003) suggests that overlap at 83 % confidence intervals indicates whether samples are significantly different, at $p = 0.05$.

Sediment core data (vertical variability) could not be successfully transformed and so a Mann-Whitney U test was used to identify significant differences between the mean from two sample groups (i.e. restored and natural samples).

4.2 Results

4.2.1 Mercury concentrations and spatial variability

Sediments within the six sampling sites contained levels of THg between 11 and 1265 ng g^{-1} (all THg and MeHg concentrations are expressed per dry mass of sediment), with the values at Ferry Lane (mean of 580 ng g^{-1}) almost three times higher than those at Orplands (mean of 190 ng g^{-1}; Table 4.2; Figure 4.1). In the mixed-effect model for THg, site type (restored, natural) was highly significant, with concentrations in natural sites consistently higher than those in restored sites (Table 4.2 and 4.3). The mean concentration of MeHg in the sediment ranged from 816-1832 pg g^{-1} (Table 4.2) and showed less variability across sites, with the highest concentrations in the natural saltmarsh at Orplands (mean > 1800 pg g^{-1}). The mixed-effect model showed a strong interaction between type and site, with no consistent pattern (Table 4.3). Physical sediment properties showed systematic variation across sites (Figure 4.1), and the mixed-effect models indicated significant interactions, with restored sites showing higher variability between sites than natural sites (Appendix 2, Figure 4.1). Northey showed the highest levels of LOI (> 17 %) and the lowest moisture contents (35.7 %) and bulk densities (0.37 g cm^{-3}) whereas the restored saltmarsh at Orplands showed the highest moisture contents (56.1 %) and bulk densities (0.71 g cm^{-3}).

The spatial scales of variation of THg, MeHg and physical sediment properties differed. For THg concentrations in both natural and restored wetlands, the vast majority of the variation was at the regional scale between sites (Table 4.5), whereas variability at the finest spatial scale (< 1 m) was small (% RSD < 14 %; Table 4.4). For MeHg and soil physico-chemical properties, the scales of variation were distinctly different for the different site types. Most of the variation in MeHg for natural saltmarshes was within sites, predominantly at the intermediate scale (~15-50 m), whereas the variation in restored wetlands was evenly split amongst the three different scales between and within sites (Table 4.5). For the physical sediment properties (LOI, moisture content, bulk density), most of the variation in natural saltmarshes was at the intermediate scale within sites (~15-50 m), whereas that for restored wetlands was between sites (Table 4.5). Fine-scale variability (< 1 m) in these properties was consistently small (%

RSD < 9 %; Table 4.4). These results indicate that THg concentration is controlled primarily by factors operating at a regional scale, whereas MeHg and the physical sediment parameters are controlled by factors that differ across natural and restored wetlands.

Table 4.2

Average total Hg (THg) and methylmercury (MeHg) sediment concentrations and physical characteristics. Range is given in parentheses.

	Total Hg ($ng\ g^{-1}$)		MeHg ($pg\ g^{-1}$)		% MeHg		LOI (%)		Moisture Content (%)		Bulk Density ($g\ cm^{-3}$)	
Orplands												
Restored	170	(112-235)	816	(0.005-2007)	0.48	(0.001-1.20)	10.7	(7.92-13.5)	56.1	(46.7-64.4)	0.71	(0.28-0.92)
Natural	211	(151-288)	1832	(389-3899)	0.83	(0.18-1.98)	15.3	(11.4-19.5)	42.5	(37.3-47.9)	0.46	(0.23-0.59)
Ferry Lane												
Restored	523	(252-742)	1146	(832-2142)	0.21	(0.13-0.37)	10.3	(8.28-13.5)	47.6	(38.5-54.0)	0.63	(0.47-0.74)
Natural	638	(432-1265)	1380	(725-2890)	0.22	(0.07-0.48)	14.9	(10.4-18.8)	44.0	(38.7-55.0)	0.46	(0.21-0.75)
Northey												
Restored	328	(159-433)	1826	(589-3183)	0.55	(0.20-0.94)	17.5	(10.0-21.9)	34.8	(27.1-51.5)	0.34	(0.14-0.65)
Natural	407	(304-482)	1201	(0.30-2809)	0.27	(0.00-0.65)	17.1	(10.5-21.7)	36.7	(29.7-49.3)	0.40	(0.18-0.67)

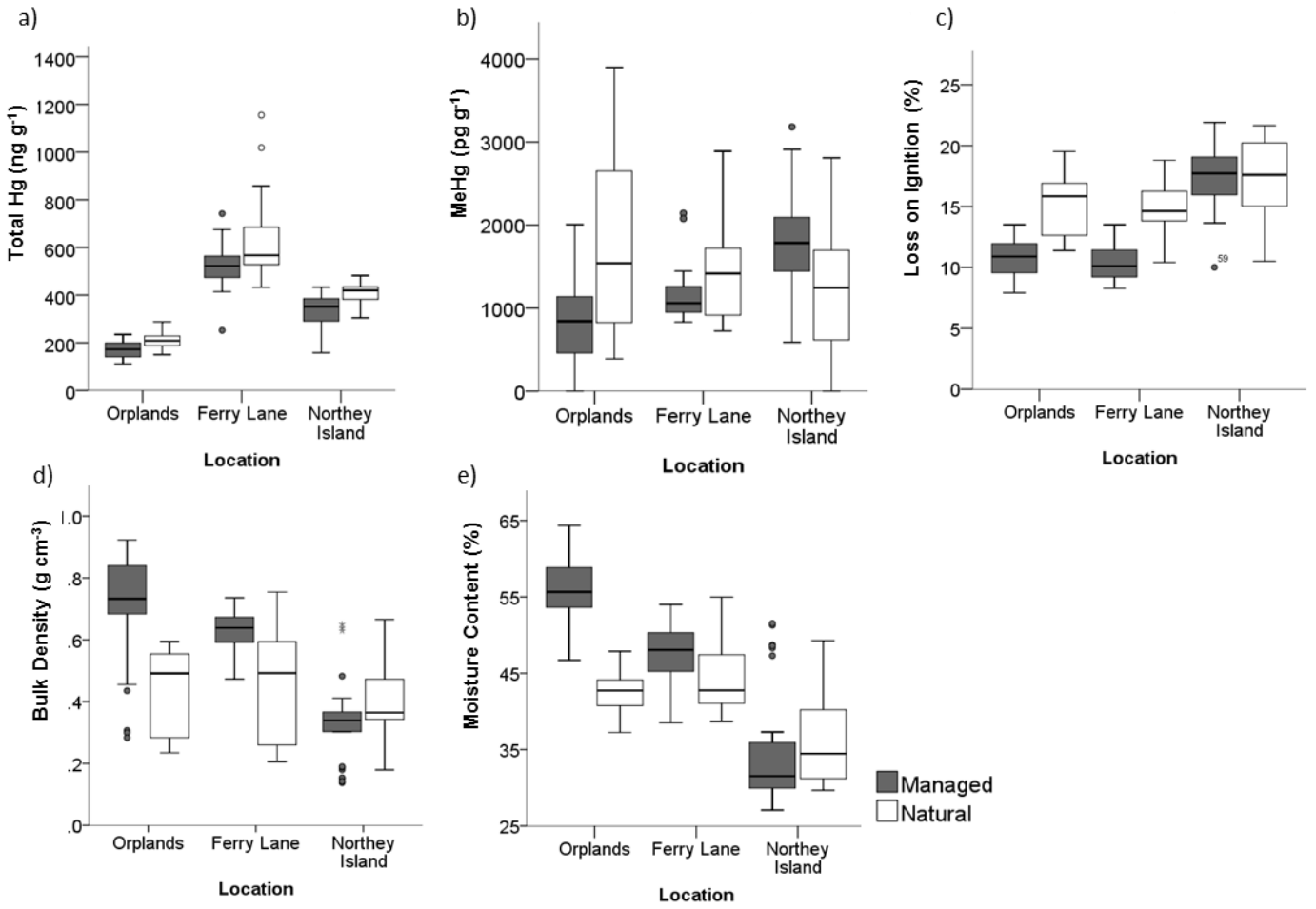

Figure 4.1. (a) Total mercury (THg; ng g^{-1}), (b) methylmercury (MeHg; pg g^{-1}), (c) loss on ignition (%), (d) bulk density (g cm^{-3}) and (e) moisture content (%) for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 4.3

Results for mixed effects models for THg and MeHg, as well as physical sediment properties. Significance tests are included for fixed effects only (not significant, ns).

Source of variation	d.f.	Mean square	F	p-value
THg content				
Type (Natural, Restored)	1	0.397	63.2	<0.001
Type X Site interaction	2	0.003	0.46	(ns)
Site (Orplands, Ferry, Northey)	2	3.51		
Location (within site)	27	0.020		
Replication (within location within site)	147	0.006		
MeHg content				
Type (Natural, Restored)	1	0.326	4.9	<0.05
Type X Site interaction	2	2.24	33.7	<0.001
Site (Orplands, Ferry, Northey)	2	0.236		
Location (within site)	27	0.211		
Replication (within location within site)	147	0.067		
Loss on ignition				
Type (Natural, Restored)	1	0.088	131	<0.001
Type X Site interaction	2	0.028	41.7	<0.001
Site (Orplands, Ferry, Northey)	2	0.082		
Location (within site)	27	0.003		
Replication (within location within site)	147	0.001		
Moisture content				
Type (Natural, Restored)	1	0.117	95.4	<0.001
Type X Site interaction	2	0.094	77.0	<0.001
Site (Orplands, Ferry, Northey)	2	0.313		
Location (within site)	27	0.012		
Replication (within location within site)	147	0.001		
Bulk density				
Type (Natural, Restored)	1	0.676	62.0	<0.001
Type X Site interaction	2	0.394	36.1	<0.001
Site (Orplands, Ferry, Northey)	2	0.806		
Location (within site)	27	0.072		
Replication (within location within site)	147	0.011		

Table 4.4

Percent relative standard deviation (%RSD) of triplicate samples taken within 1m of each other

Location	Total Hg	MeHg	LOI	Moisture Content	Bulk Density
Orplands					
Restored	8.3	54.5	5.9	3.2	7.6
Natural	7.8	24.7	5.9	2.0	6.8
Ferry Lane					
Restored	9.8	14.2	6.7	1.6	2.8
Natural	13.4	17.3	8.4	2.7	8.7
Northey					
Restored	6.6	38.7	7.9	2.6	7.8
Natural	10.5	20.7	6.2	1.5	5.8

Table 4.5

Variance partitioning for sediment total mercury and methylmercury content, as well as physical sediment parameters. See method of analysis for further explanation.

Source of Variation	Natural		Restored	
	d.f.	% of variance	d.f.	% of variance
THg content				
Site (Orplands, Ferry, Northey)	2	85	2	78
Location (within site)	27	7	27	17
Replication (within location within site)	60	7	60	5
MeHg content				
Site (Orplands, Ferry, Northey)	2	1	2	37
Location (within site)	27	73	27	32
Replication (within location within site)	60	26	60	32
Loss on ignition				
Site (Orplands, Ferry, Northey)	2	2	2	72
Location (within site)	27	78	27	19
Replication (within location within site)	60	20	60	8
Moisture content				
Site (Orplands, Ferry, Northey)	2	25	2	68
Location (within site)	27	71	27	30
Replication (within location within site)	60	4	60	2
Bulk density				
Site (Orplands, Ferry, Northey)	2	<1	2	55
Location (within site)	27	83	27	38
Replication (within location within site)	60	17	60	7

The vertical distribution of THg and MeHg concentration in triplicate sediment cores at Orplands natural and restored site are illustrated in Figure 4.2. The THg sediment profile in the natural site exhibited high variation between cores. The surface sediments contained the lowest THg concentrations (59-142 ng g^{-1}) and increased with depth to a maximum of 210-260 ng g^{-1} at 9 cm depth, and then decreased to 59-142 ng g^{-1} at 25 cm depth. THg concentrations in the natural site were significantly higher than in the restored site (Mann-Whitney U = 65, $p<0.001$). The THg sediment profiles in the restored site contained maximum concentrations in the surface sediment (0-4 cm, 140-195 ng g^{-1}) and decreased with depth to a minimum of 35-45 ng g^{-1} at 25 cm depth. All three cores showed a similar profile with little variation between cores.

The MeHg sediment profiles (Figure 4.2) showed much higher variation between cores than the THg profiles. MeHg sediment profiles in the natural site were lowest at the surface (40-130 pg g^{-1}) but MeHg concentrations increased to maximum concentrations of between 208 and 300 pg g^{-1} at 7-19 cm depth. MeHg concentrations then decreased with depth in all three cores. MeHg concentrations in the natural site were significantly lower than in the restored site (Mann-Whitney U = 378, $p<0.001$). In the restored site MeHg concentrations were also lowest in the surface sediment (24-180 pg g^{-1}). MeHg concentrations increased to a maximum of 980 pg g^{-1} at 13 cm depth before decreasing to approximately 350 pg g^{-1} at 25 cm depth. Peaks in MeHg concentration occurred between 13 and 17 cm depth in all three cores and decreased at lower depths.

MeHg as a percentage of THg (%MeHg) with depth is presented in Figure 4.3. The % MeHg profile in the natural saltmarsh showed variable profiles with depth. The surface sediments contained the lowest % MeHg values, between 0.4 and 0.75 %, and increased with depth, although peaks in % MeHg occurred at difference depths in different cores. The % MeHg profiles for the restored site were significantly higher than the natural site (Mann-Whitney U = 79, $p<0.001$). The % MeHg profiles in the restored site were also lowest in the surface sediment (0.2-1.3 %). Values increased

with depth to a maximum of 3 to 13 % at 13 to 17 cm depth and then decreased at lower depths.

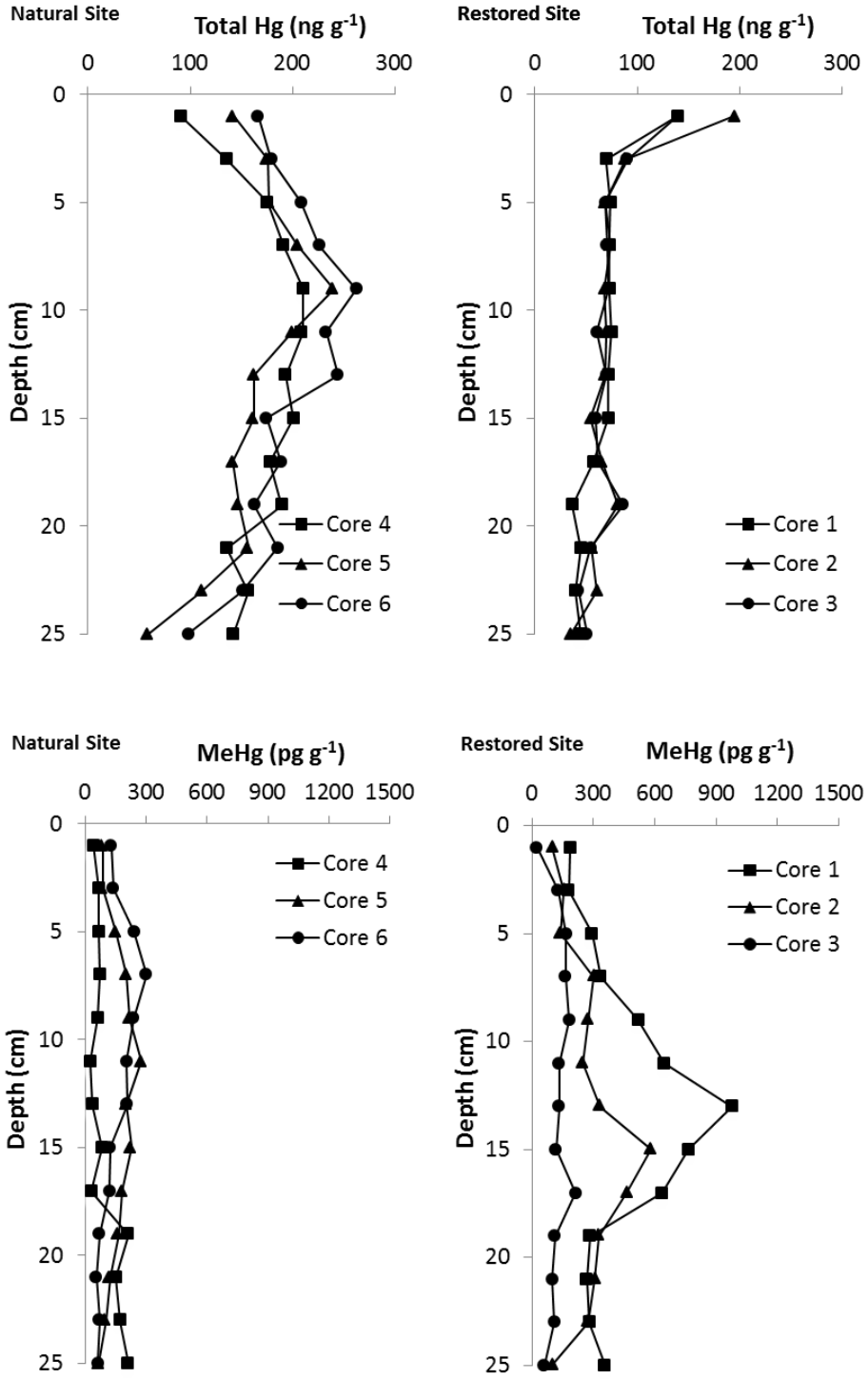

Figure 4.2 Sediment THg and MeHg profiles from Orplands restored and natural saltmarsh. The standard errors, not shown for aesthetic reasons, are small in the natural site for both THg (c. 15) and MeHg (c.45) and the restored site for THg (c. 5, smaller than the size of the symbol). The standard error for MeHg in the resorted site was 100, approximately 1.5 times the size of the symbols.

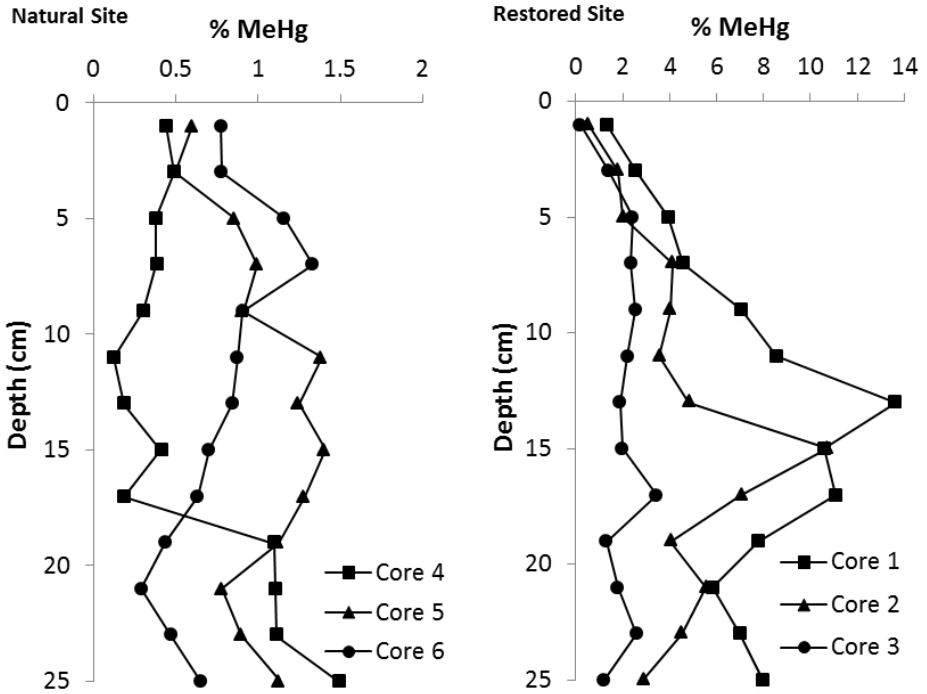

Figure 4.3 % MeHg sediment profiles from Orplands restored and natural saltmarsh. The standard errors, not shown for aesthetic reasons, are small in the natural site (c. 0.24) and restored site (c. 1.56).

4.2.2 Association amongst mercury and other physico-chemical parameters

Correlations between THg, MeHg and physical sediment characteristics are given in Table 4.6. Total Hg was not correlated with LOI or bulk density (Figure 4.4a), but had a weak negative association with moisture content (r=-0.247, p<0.001). Scatterplots (Figure 4.4a) for THg and physico-chemical variables identified two separate groups of data, one group for Ferry Lane and another for both Orplands and Northey Island, suggesting that sources and/or behaviour of THg differed across the three sites. Once data for Ferry Lane were removed from the analysis, there were strong correlations between THg and moisture content (r =-0.713, p<0.001), bulk density (r =-0.466, p<0.001) and LOI (r =0.576, p<0.001). No correlation was found between THg and MeHg concentrations. MeHg showed a significant positive correlation with LOI (r=0.388, p<0.001), and a significant negative association with bulk density (r=-0.299,

p<0.001) and moisture content (*r*=-0.445, *p*<0.001). A weak correlation was present between % MeHg and LOI (*r*=0.213, *p*<0.01). No correlation was found between % MeHg and bulk density and moisture content (figure 4.4b).

Table 4.6
Spearmans rank correlations between sediment mercury concentration and
sediment characteristics. P-values are given in parentheses.

	Loss on Ignition		Bulk Density		Moisture Content	
THg	0.035	(0.640)	-0.105	(0.162)	-0.247	(0.001)
MeHg	0.388	(0.001)	-0.299	(0.001)	0.445	(0.001)
% MeHg	0.213	(0.004)	-0.115	(0.123)	-0.113	(0.132)

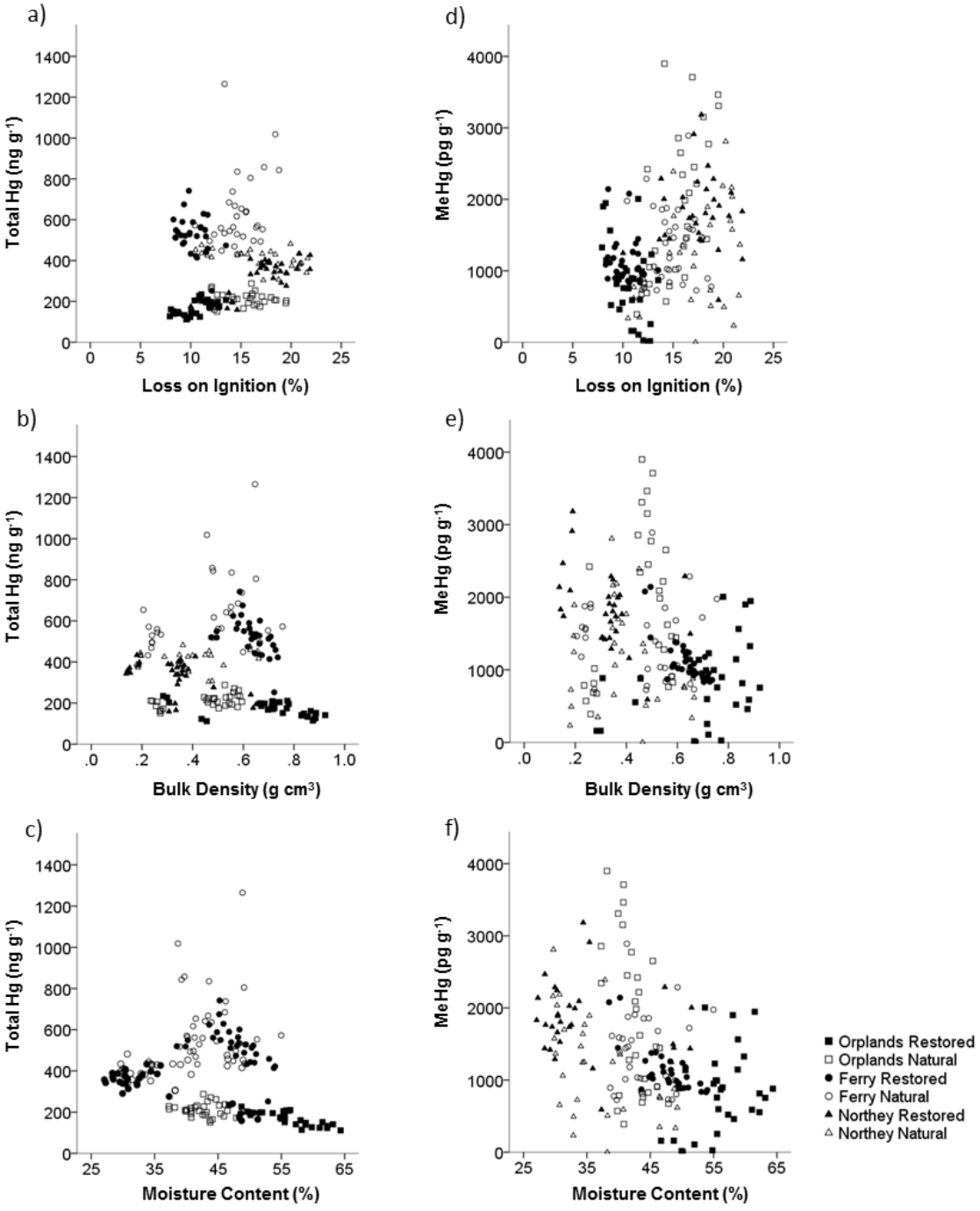

Figure 4.4a. Bivariate plots of THg against (a) loss on ignition, (b) bulk density and (c) moisture content, and MeHg against (d) loss on ignition, (e) bulk density and (f) moisture content.

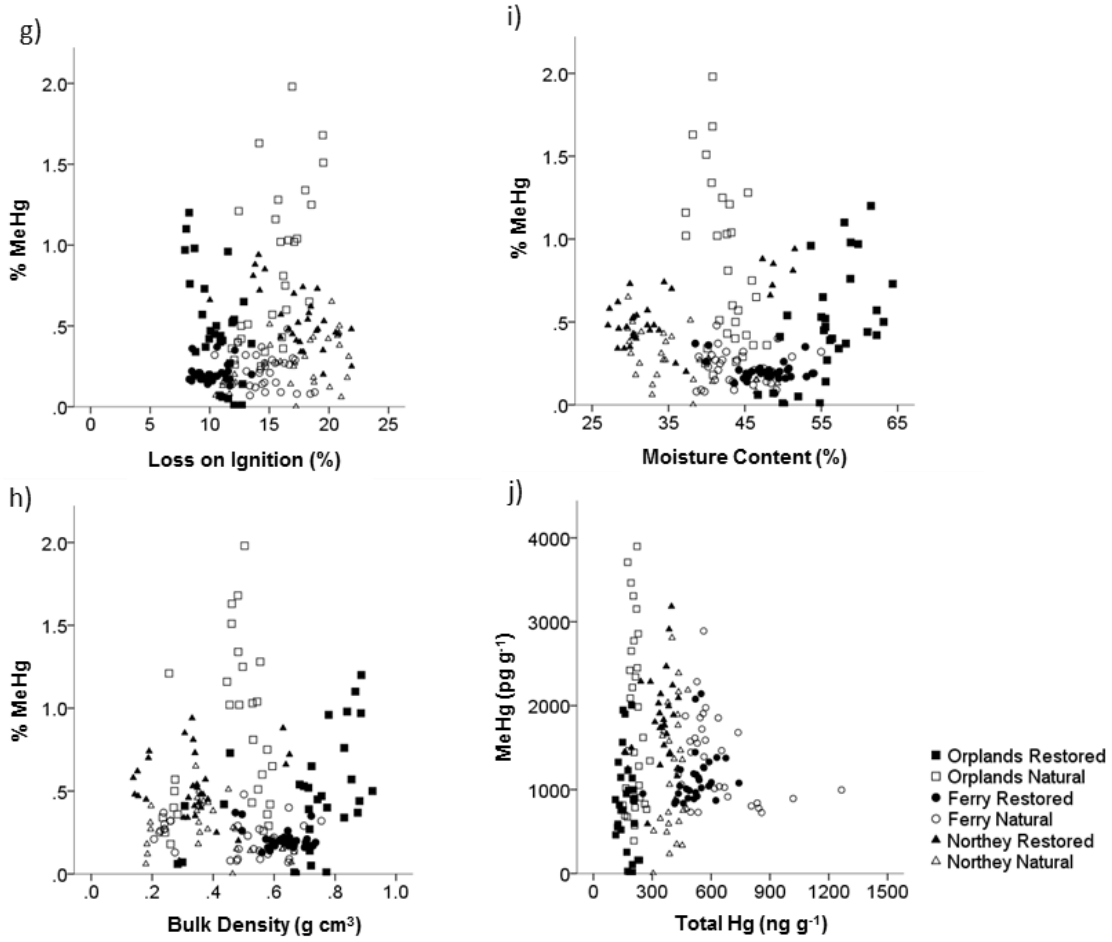

Figure 4.4b. Bivariate plots of % MeHg against (g) loss on ignition, (h) bulk density and (i) moisture content and MeHg against (j) THg

4.2.3 Trends in mercury concentrations since de-embankment

Total Hg concentrations showed no trend with time since de-embankment, and concentrations in restored sites were slightly lower than those in natural saltmarshes (i.e., THg differences were less than zero) (Figure 4.5a). Inventories to 30 cm depth were calculated for the composited cores to quantify the amounts of Hg present and to enable broad-scale comparison between natural and restored sites where sediment bulk densities vary. Total Hg inventories showed that recently-restored sites stored similar amounts of THg as adjacent natural saltmarshes, whereas the site of earliest de-embankment (Northey) stored less than its natural counterpart (Figure 4.5c). MeHg

concentrations in the most recently-restored site (Orplands) were lower than in the adjacent natural site (an average negative difference of 1 ng g^{-1}), but increased with time since de-embankment such that the earliest-restored site (Northey) showed slightly higher concentrations than in the natural saltmarsh (an average positive difference of 625 pg g^{-1}) (Figure 4.5b). MeHg inventories in restored sites showed a step-like increase with time since de-embankment, with storage in the oldest sites (Ferry, Northey) slightly higher than in adjacent natural saltmarshes (Figure 4.5d). LOI showed a similar increasing trend (Figure 4.6a), with values at the oldest restored site (Northey) equal to those in the adjacent saltmarsh. Moisture content and bulk density showed a decreasing trend, again with values at the oldest restored site (Northey) equal to those in the adjacent saltmarsh (Figure 4.6b and c). These findings indicate that, by 115 years after de-embankment, MeHg concentrations and physico-chemical parameters in the restored sites had converged with values similar to those in natural saltmarshes. In contrast, THg concentrations were unrelated to the temporal development of restored sites.

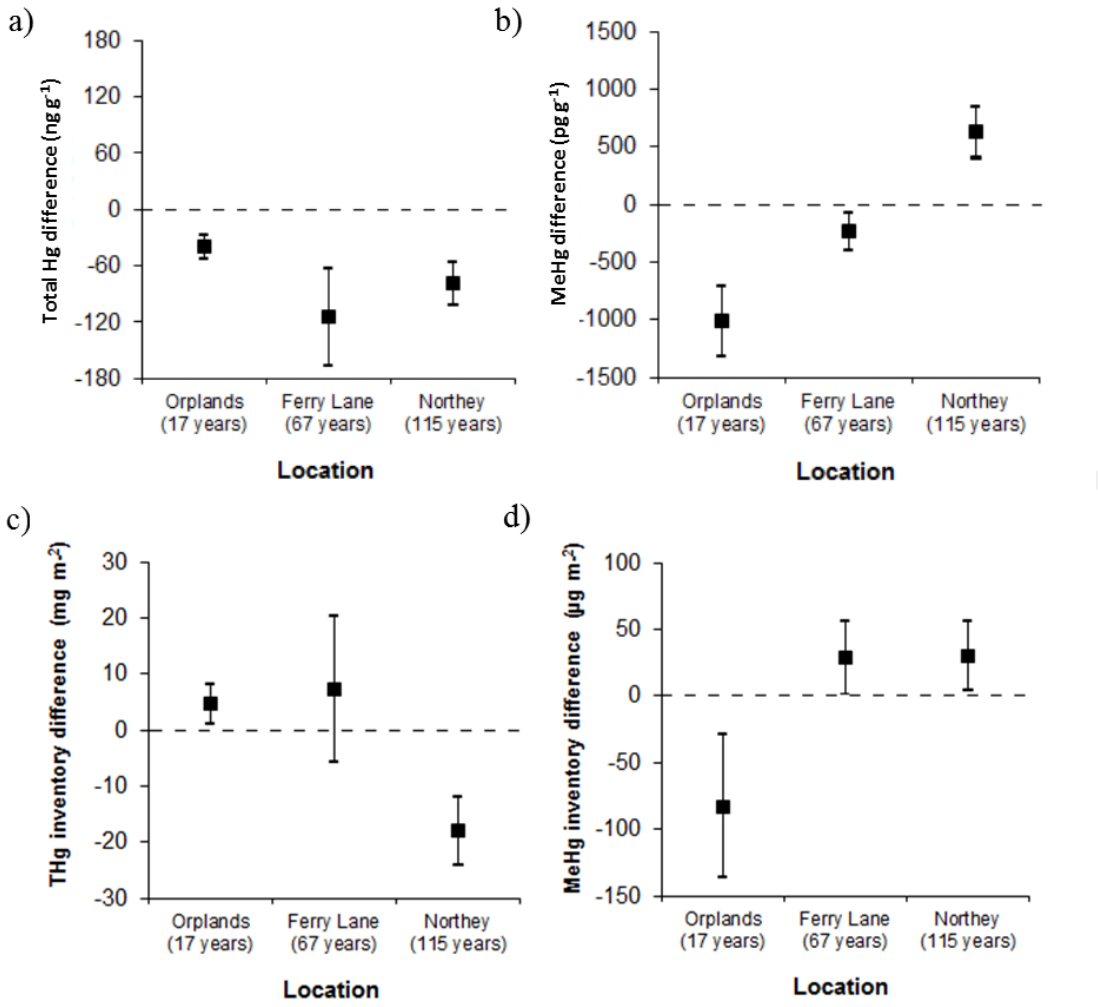

Figure 4.5. Difference between natural and de-embankment sites (de-embankment minus natural) for (a) THg, (b) MeHg, (c) THg inventory and (d) MeHg inventory for Orplands, Ferry Lane, and Northey Island representing time since de-embankment (errors were propagated to give 83 % confidence intervals; see text in Section 4.1.2 for more details).

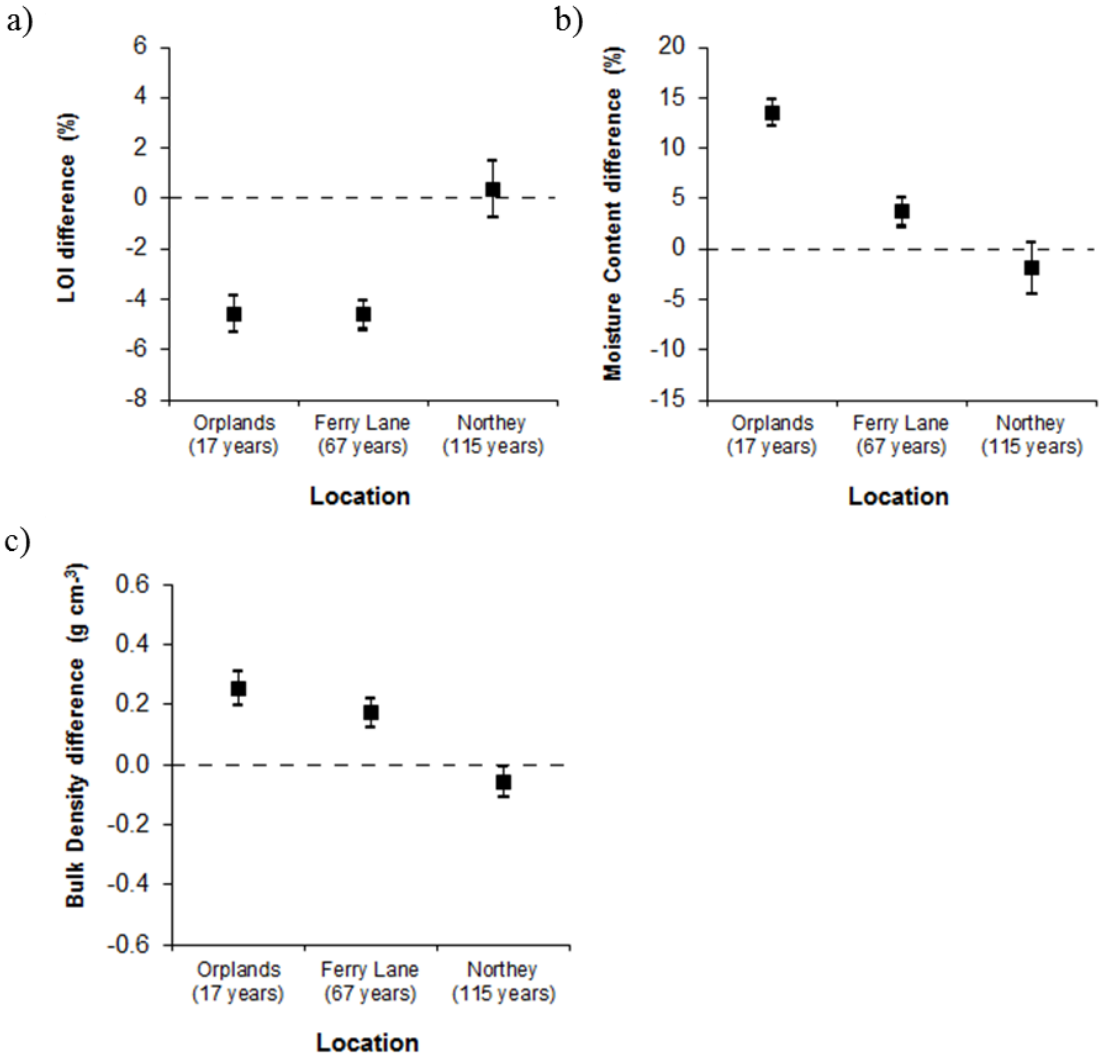

Figure 4.6. Differences between natural and de-embankment sites (de-embankment minus natural) for (a) loss on ignition, (b) moisture content and (c) bulk density for Orplands, Ferry Lane, and Northey Island representing time since de-embankment (errors were propagated to give 83 % confidence intervals; see text in Section 4.1.2 for more details).

4.3 Discussion

4.3.1 Mercury spatial variability and association with physico-chemical parameters

4.3.1.1 Total Hg

Overall, sediments within the six de-embanked and natural sites had high concentrations of THg compared to other published data for estuarine systems (e.g. Sunderland *et al*. 2006, Hung and Chmura 2006, Conaway *et al*. 2003, Emmerson *et al*. 1997, Hammerschmidt *et al*. 2004, Choe *et al*. 2004; Table 2.1). All six sites had sediment THg concentrations higher than the Threshold Effect Level (TEL) of 130 ng g^{-1}, stated by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment 1995). Ferry Lane contained THg concentrations higher than the Probable Effect Level (PEL) of 700 ng g^{-1} (Canadian Council of Ministers of the Environment 1995). The Threshold Effect Level (TEL) of 130 ng g^{-1} is the value developed by the Canadian Council of Ministers of the Environment as a broadly protective tool to support the functioning of healthy aquatic ecosystems. It states that Hg levels above 130 ng g^{-1} adverse biological effects occasionally occur.

Strong negative correlations between moisture content and THg were observed, epically once Ferry Lane data were removed. Oxygen is utilised within a few millimeters of the surface in waterlogged sediment and therefore the next electron acceptor is used by microorganisms in order to respire (Kostka *et al*. 2002). Consequently, saltmarsh sediments are characterised by redox-stratified sediments. Any metals, such as THg, complexed to these alternative electron acceptors are released into porewater (Otero *et al*. 2009). Metals in their dissolved phase are significantly more mobile and bioavailable than solid-phase metals (discussed within the literature review, Section 2.5) and their concentration in sediment will be reduced.

The results indicate that controls on THg were primarily controlled by site-specific factors, rather than time since de-embankment, within-site heterogeneity or physico-chemical sediment parameters. However, when Ferry Lane, the most heavily contaminated site, was removed from the analysis, THg concentrations did have strong

correlations with physico-chemical parameters. Atmospheric deposition (from e.g. fossil fuel combustion and smelting) is an important source of Hg to aquatic coastal environments and due to the long residence time and transport distances of atmospheric Hg (Driscoll *et al*. 2013), the sites in this study are likely to receive similar atmospheric inputs, particularly in the absence of regional industries known to emit Hg (e.g. smelting). Saltmarshes may also receive Hg inputs from surface floodwaters from e.g. mining, agricultural application of organomercurials, and industrial and domestic wastewaters (Davis *et al*. 2001, Ullrich *et al*. 2001) and the differences in THg sediment concentrations may reflect proximity to local Hg sources, with the Colne River (Ferry Lane) receiving higher floodwater Hg loads than the other sites. All three sites are downstream from sewage treatment works (STW) and although additional Hg sources upstream from Ferry Lane have not been identified, this site is proximal to an old shipping dock and historic use of antifouling paints may be a local source of Hg contamination (Berto *et al*. 2006). A STW that had a significant pollution incident in 2011 involving fuel and power as well as water pollution is also 2.5 km upstream to the Ferry Lane sites (Environment Agency 2011).

The vertical THg profiles for the natural saltmarsh at Orplands are typical of a vegetated saltmarsh (Mitchell and Gilmour 2008). THg concentrations peak at 9 cm depth which corresponds to approximately 1967 (Spencer *et al*. (2008) estimated Orplands natural saltmarsh had an average sedimentation rate of 0.2 cm a^{-1}). The decrease in THg concentration from the peak to the surface may indicate improved water quality in the Blackwater estuary and reductions in THg emissions from coal burning power plants. At depth, concentrations are decreasing, although at 25 cm depth concentrations have not yet levelled out and so are most likely still above background levels.

The THg profiles for the restored site at Orplands are more typical of a mudflat or unvegetated coastal sediments (Yee *et al*. 2008, Hammerschmidt *et al*. 2004). The vertical distribution of THg does not show any appreciable vertical variability, except

with higher THg concentrations in the surface sediment and concentrations decreasing slightly with depth. Spencer *et al*. (2008) estimated Orplands restored site had an average sediment accumulation rate post-breach of approximately 0.75 cm a^{-1}. Therefore in 2012 it is expected that the pre-breach land surface (1995) would be between 10.75 and 12.75 cm depth, although it is important to note that accumulation rates would have decreased with time as the marsh surface reaches equilibrium. The cores were also taken from different locations within the marsh compared to Spencer *et al*. (2008) which would affect sediment accumulation rates. Therefore, these accumulation rates are used with caution. There is no evidence of the pre-breach surface horizon in the THg profile. Apart from the peak near the surface, no peaks are evident within the profile suggesting that either the sediment is well-mixed or that the sediment was not exposed to varying Hg inputs. At depth (25 cm) concentrations average 40 ng g^{-1} which are likely to be close to geochemical background levels (Yang and Rose 2003).

THg concentrations are also lower in the restored site compared to the natural saltmarsh. There are three possible reasons for these differences. Firstly, the restored saltmarsh, contains less organic matter which has a significant control on the storage on THg in sediments (Bryan and Langston 1992). Saltmarshes store Hg from past anthropogenic activity and atmospheric input due to their fine-grained and organic-rich sediments (Ullrich *et al*. 2001) and so the saltmarsh sediments, which have significantly more LOI than the restored site (Figure 4.1c), are able to trap more THg entering the saltmarsh via tidal inundation. Secondly, the restored site has been disconnected from tidal inundation since its embankment in the 18th century (Wolters *et al*. 2005). Therefore, the only source of Hg to these sediments would have been atmospheric deposition. Thirdly, the managed realignment site used to be agricultural land before being reconnected to tidal inundation. Agricultural land has been shown to be a source of atmospheric Hg during tillage (Bash and Miller 2007) resulting in the loss of Hg to the atmosphere.

4.3.1.2 MeHg

Concentrations of MeHg in the sediment were moderate to high compared with other studies reported in the literature (Conaway *et al*. 2003; see Table 2.1). In natural saltmarshes, MeHg concentrations varied predominantly at the intermediate (~15-50 m) spatial scale, as did the physico-chemical parameters. This scale of variation reflects topographic and microhabitat heterogeneity (Larkin *et al*. 2006). Bulk density, moisture content and LOI are inter-related and Allen (2000) showed the multiple feedbacks between saltmarsh primary productivity (a key control of organic matter content), autocompaction (density), hydroperiod and marsh elevation. Organic matter decomposition has also been shown to vary spatially with salinity and elevation (Craft 2007). Vegetation will vary spatially from the seaward to landward edge of the saltmarsh and plant roots exude labile organic carbon in the rhizosphere which stimulates microbial activity and hence methylation (Windham-Myers *et al*. 2009). The significant correlations between MeHg concentrations and physico-chemical properties, as well as the similarity in spatial scales of variation, suggest that sediment physico-chemical conditions and/or vegetation patterns related to this within-site heterogeneity exert control on Hg methylation.

For de-embanked sites, physical sediment properties varied mainly between sites and reflected saltmarsh development with time since breach. MeHg concentrations tracked this development, but MeHg varied more within restored sites (particularly at fine scales) than did the physico-chemical parameters. In this study, within site sample locations were selected at random with no control for biogeomorphological variability. For the restored sites, the small within-site variability in the physico-chemical parameters suggests that they have less biogeomorphological variability than the natural sites, as seen in other restoration studies (Elsey-Quirk *et al*. 2009). There is perhaps a parameter controlling Hg methylation that varies spatially at a fine-scale that has not been measured in this study, e.g. salinity, sulphide, or pH (Compeau and Bartha 1984, Benoit *et al*. 1999, Ullrich *et al*. 2001).

In the vertical cores from Orplands site, MeHg concentrations were significantly lower in the natural saltmarsh than in the restored site. This finding contrasts with that from the composited core data (Objective 1; Figure 4.1b), in which the restored site contained significantly less MeHg than the natural saltmarsh. The sampling strategy used for objective 1 was designed so that the results were representative of the entire site. However the sampling strategy used for objective 2 was designed so that the cores were paired in elevation and hence tidal regime. Cores were collected at the landward edge of the restored site where sufficient sediment accumulation has taken place so that the elevation of the site is equal to the natural site. Therefore, for objective 2, elevation and tidal inundation are controlled so differences in MeHg concentrations between restored and natural sites are due to differences in sediment properties arising from past land-use. Tempest *et al*. (2014) show that sediment properties and hydrology are very different in the restored saltmarsh with reduced water-level fluctuations and poor vertical hydrological connectivity in the restored saltmarsh.

MeHg concentrations and % MeHg in the natural site were at the lower range of values reported from similar studies whereas concentrations in the restored site are at the mid to higher range of values reported (Mitchell and Gilmour 2008). In the restored site, the MeHg concentrations and % MeHg generally start to increase at depths below 10 cm. Below 10.75-12.75 cm depth (estimated pre-breach land surface) it is likely that the sediment would still retain the properties characteristic of a compacted agricultural soil. The sediment has higher bulk density (Figure 4.1d) because of legacy effects of past land-use including compaction, carbon mineralisation and dewatering (Wolters *et al*. 2005) which results in poorly drained sediments. Compaction of the agricultural soil may inhibit vertical porewater movement and tidal flushing. Therefore, de-embankment and saline inundation creates waterlogged sediment (Figure 4.1e), which in turn can promote the development of reducing conditions and increased salinity, all of which are favourable conditions for SRB and Hg methylation. These differences in physico-chemical properties in the restored site are likely to have

produced a peak in MeHg concentrations at 13 cm depth at significantly higher concentrations than the natural saltmarsh sediments.

Comparing depth integrated cores from these two sites (restored and natural) at Orplands gives a clear indication of differences in MeHg concentrations between natural saltmarshes and restored wetlands because the cores were paired and so equal in tidal regime. By collecting cores at similar elevation, the data gives an indication of future MeHg concentrations that can be expected to develop in the restored saltmarsh overtime as the surface elevation increases. For example, MeHg concentrations in the composited cores taken from the restored site at Northey Island were also significantly higher than composited cores from the natural site (Figure 4.5b). It is likely that de-embanked sites, once fully restored, are likely to produce higher concentrations of MeHg than their natural counterparts due to the permanent physico-chemical changes that have occurred in the sediment. The physico-chemical changes associated with past land-use and subsequent restoration promote Hg methylation, leading to MeHg concentrations in restored wetlands higher than in their natural counterparts. Therefore, saltmarsh restoration could potentially create wetlands that produce significantly higher MeHg concentrations than their natural counterpart and have a detrimental impact on the wildlife living in the saltmarsh and the wider estuary.

4.3.2 Physico-chemical conditions, total mercury and methylmercury with time since breach

Saltmarsh restoration leads to a series of physical changes that may impact on sediment biogeochemistry. Prior to de-embankment, the surface elevation is low in comparison with the surrounding adjacent natural saltmarsh. The sediments also have high bulk density, low organic matter content and low moisture content compared with sediment in natural saltmarshes due to pre-restoration land use and drainage (French, 2006). The legacy effects of past land-use (agriculture and drainage) including compaction, carbon mineralisation and dewatering (Wolters *et al*. 2005) results in

poorly drained sediments. These compacted sediments impede drainage, resulting in water-logged conditions in the upper sediment layer (Crooks *et al*. 2002, Tempest *et al*. 2014, Spencer *et al*. 2008). Therefore, de-embankment and saline inundation can create anoxic sediments, which in turn can promote the development of reducing conditions, reduced pH and increased salinity. These changes in sediment conditions can alter the mobility of contaminant metals (Emmerson *et al*. 2001, Teuchies *et al*. 2013, Speelmans *et al*. 2010). Changes in salinity can alter the partitioning of metals between solid and aqueous phases which occur through two main processes. Firstly, desorption regulated by complexation with saltwater anions (eg. Cl^- and SO_4^{2-}) and secondly competition for sorption sites with cations (Na^+, K^+, Ca^{2+} and Mg^{2+}). An increase in salinity is associated with an increase in major cations that compete with heavy metals for the sorption sites (Du Laing *et al*. 2009). Therefore, inundation with saline water can cause the dissolution of minerals and results in the release of trace metals into the aqueous phase (Speelmans *et al*. 2007). Flooding soils, independent of salinity, can also change metal availability due to the decrease in redox potential (Speelmans *et al*. 2007). A reduction of Fe and Mn oxides result in the release of associated metals, such as Hg.

The legacy effect of land use on sediment properties is reflected in the high bulk density and moisture content for the most-recently restored site (Orplands), where the pre-restoration land surface was likely to be only 10-20 cm below the current saltmarsh surface (Spencer *et al*. 2008). Newly restored saltmarshes have been shown to have less topographic and microhabitat heterogeneity (Elsey-Quirk *et al*. 2009) and their vegetation is not equivalent to their natural counterparts in terms of species abundance and diversity (Mossman *et al*. 2012, Garbutt and Wolters 2008). In the decades following de-embankment, physico-chemical properties in restored sites converge towards those in the natural saltmarshes (Figure 4.6). As the restored sites accumulate sediment, surface elevation increases, reducing the hydroperiod and allowing sediment to drain more effectively, enabling saltmarsh plants to colonise and the establishment of saltmarsh vegetation (Garbutt and Wolters 2008). These changes

are reflected in the increasing LOI content, and decreasing bulk density and moisture content observed across the sites with increasing time since breach.

THg concentration did not show any relationship with time since breach, supporting the conclusions of the variance partitioning analysis and associations between THg and physico-chemical parameters, i.e., that THg concentrations were strongly influenced by external sources. The de-embanked sites had lower THg concentrations than the adjacent natural saltmarshes, and this may be due to lower Hg inputs, higher inputs of relatively uncontaminated sediment, differences in sediment density, and/or loss of gaseous Hg from the system due to tillage (Bash and Miller, 2007). For example, saltmarsh cores frequently provide a record of Hg input reflecting anthropogenic activity from the late 1800s to the mid-20th Century (Fox *et al*. 1999). This is evident in the THg vertical profile cores taken from Orplands natural saltmarsh. For much of this time the restored sites were disconnected from tidal/fluvial inputs (embanked and drained for agriculture) and therefore THg concentrations averaged over the sediment core may be lower in restored sites particularly if fluvial inputs were significant Hg sources. The restored sites would have also experienced rapid sediment accumulation following de-embankment diluting any atmospheric Hg input. Inorganic Hg is delivered to the saltmarsh surface via tidal inundation and/or atmospheric deposition where it is either reduced to Hg^0 or scavenged and buried with sediment. Consequently total Hg (THg) sediment concentrations are frequently elevated at depth reflecting past air and water quality, although profiles can vary significantly depending on sedimentation rates and post-depositional physical and chemical reworking (e.g. Hammerschmidt *et al*. (2004); Mitchell and Gilmour (2008)).

Total Hg inventories are only significantly lower in the site which was de-embanked over 100 years ago (Northey) suggesting that differences in sediment density may partly account for these lower THg concentrations in more recently de-embanked sites (Figure 4.5). This could also reflect the timing of the high sedimentation rates the sites would have experienced following de-embankment. In addition, adsorption of Hg[II] to

organic matter has been identified as an important mechanism that facilitates the transport, as well as the mobility and bioavailability, of Hg within the aquatic environment (Bryan and Langston 1992). Lower LOI content in Orplands and Ferry Lane restored sites could also explain the low THg concentrations. Without dated vertical profiles of sediment THg concentration it is difficult to elucidate these differences further.

Concentrations and inventories of MeHg increased with time since de-embankment, indicating that restored sites could potentially become hotspots for MeHg production and storage. The trend of MeHg concentrations increasing with time since de-embankment has three possible explanations. Firstly, THg concentration may control Hg methylation. However, there was no clear relationship between MeHg and THg (Figure 4.4), indicating that inorganic Hg is either not the limiting factor for Hg methylation or is not uniformly bioavailable. Other studies have also found no relationship (e.g. Ullrich *et al.* 2001, Conaway *et al*. 2003, Lambertsson and Nilsson 2006), highlighting the importance of biogeochemical controls on Hg methylation.

A second explanation for the increasing trend of both MeHg concentration and inventory with time since de-embankment may be that the sediment is accumulating MeHg over time, without any change in Hg methylation rate. Therefore, any variation in MeHg concentrations will be a function of sedimentation rates or bulk density of the sediment. Methylmercury binds strongly to sulphur species and organic matter, and so as the sulphur and organic matter content of the sediment increases over time more methylmercury may be stored in the sediment. However, there were no differences for sediment elemental sulphur across sites or between natural and restored sites (data not shown).

A third possible explanation is that Hg methylation rates may be increasing over time as sediment conditions change following de-embankment and sediment conditions no longer inhibit MeHg production. In this study, MeHg concentrations changed in

tandem with physico-chemical conditions with increasing time since de-embankment, with conditions in the site with the longest history of restoration (Northey Island) approaching those in the adjacent natural saltmarsh. In recently restored sites, poor drainage may create anoxic environments with high SRB activity. Sulphide concentrations will increase if rates of sulphide removal and re-oxidation are low because of slow porewater advection and development of anoxic conditions. High sulphide concentrations create negatively charged species that are not bioavailable to methylating bacteria and so the rate of MeHg production decreases (Compeau and Bartha 1984, Benoit *et al*. 1999, Benoit *et al*. 2001). In sites that have recovered to a state close to that of natural saltmarsh, accumulated sulphides will be flushed out and re-oxidised as the sediment undergoes periodic wetting and drying. The re-oxidation of reduced sulphur can potentially make Hg[II] available to SRB (Yee *et al*. 2005). For this reason, methylation rates are greatest at the anoxic/oxic boundary, usually within a few centimetres of the surface in saltmarsh sediments decreasing with depth (Fitzgerald *et al*. 2007, Choe *et al*. 2004). However, bioturbation can increase Hg methylation at depth by removing toxic by-products of microbial respiration (e.g. S^{2-}) as well as providing labile organic substrates. Vegetation can also increase Hg methylation with plants promoting SO_4^{2-} cycling (Yee *et al*. 2005) and exuding labile organic carbon in the rhizosphere which stimulates microbial activity and hence methylation (Windham-Myers *et al*. 2009). Therefore, sediments with higher plant density can contain a larger zone of Hg methylation. Vegetation was not examined in this study. However, low LOI content and bare sediment patches were observed during field work at the most recently de-embanked site (Orplands) suggesting that vegetation cover is low in these early stages of de-embankment. Other studies (e.g. Garbutt and Wolters 2008) have also shown that differences in vegetation between restored and natural marshes decrease over time. The third explanation is most likely and therefore, Hg methylation rates are increasing over time as sediment conditions change following de-embankment and sediment conditions no longer inhibit MeHg production.

4.4 Conclusions

Total mercury and MeHg concentrations in restored and natural saltmarshes in SE England are moderate to high compared to other studies. Variability in THg concentration is predominantly controlled by external contaminant sources with high concentrations observed proximal to a historic boatyard, suggesting that the use of anti-fouling paints may be a potential Hg source.

Physical sediment properties are less heterogeneous in restored than in natural sites at the intermediate scale (15-50 m), which is reflecting lower habitat and topographic heterogeneity. This finding is in agreement with current literature and has significant implications for MeHg concentrations and variability in restored coastal wetlands. Recently de-embanked sites have lower MeHg concentrations, probably due to poor drainage and limited vegetation development. Therefore, in the first few decades following de-embankment restoration does not produce MeHg hotspots. Early stages of restoration appear to inhibit MeHg production. However, detailed vertical profiles of MeHg from paired cores indicate that once the elevation of the restored marsh has increased and the sediment is able to drain more efficiently, the restored site contains significantly higher MeHg concentrations than its natural counterpart.

In restored sites, previous land-use has a significant impact on physico-chemical sediment characteristics and these characteristics change over time to reflect saltmarsh development. Methylmercury concentrations tracked this development increasing with time, such that at Northey Island, which was de-embanked > 100 years ago, MeHg concentrations in the natural and restored site are comparable. This suggests that it takes many decades for restored sites to attain similar physical and biogeochemical characteristics to their natural counterparts. This could have significant implications for wider biogeochemical cycling in restored saltmarshes, and long-term implications for the delivery of biogeochemical ecosystem services such as water purification.

This aspect of the research was completely novel, providing the first evidence of the spatial and temporal variation of Hg and MeHg concentrations in restored saltmarshes in the UK. It is clear that physico-chemical sediment conditions affect Hg methylation. Therefore, it is important to understand further the short-term biogeochemical implications of inundating compact, poorly drained sediments (i.e. immediately following de-embankment) and to understand how restoration design e.g. controlling tidal inundation cycles (controlled reduced tide schemes), and modifying drainage and elevation might affect Hg methylation.

Chapter 5: Short-term methylmercury dynamics in re-flooded agricultural soils and implications for the tidal inundation of low-lying coastal land: a laboratory study

5.1 Introduction

Inundation with seawater causes strong biogeochemical gradients to form within the sediment profile and changes in redox (*Eh*) and salinity can potentially increase the mobility and partitioning of contaminants within the sediment. Many coastal sediments have moderate mercury (Hg) concentrations as a result of legacy industrial contamination. Despite the ecological benefits of coastal restoration, concerns have been expressed over the negative impact on wildlife and human health in mercury (Hg) contaminated areas (Morris *et al*. 2014, Yee *et al*. 2008).

Methylmercury (MeHg), a potent neurotoxin, is the mercury species of most concern because of its ability to bioaccumulate and biomagnify to toxic concentrations in food webs (Compeau and Bartha 1985, Hintelmann 2009). The methylation of a small amount of inorganic mercury (Hg[II]) can result in dangerous concentrations in fish, and mammals that consume fish (THg concentrations > 0.5 μg g^{-1} are considered dangerous), including humans (St. Louis *et al*. 1994, Hall *et al*. 2005, U.S. Food and Drug Administration 2002). Wetlands are important sources of MeHg to aquatic ecosystems (St. Louis *et al*. 1994, Hall *et al*. 2008) and therefore, increasing wetland area through restoration has the potential to produce hotspots for MeHg production (Marvin-DiPasquale and Cox 2007).

MeHg production is principally formed in anoxic sediments by sulphate reducing bacteria (SRB; Benoit *et al*. 1999) although iron reducing bacteria (FeBR) can also be important (Kerin *et al*. 2006, Mitchell and Gilmour 2008). *Eh* status and sulphate concentration are important controls on SRB and consequently are also important controls on MeHg production. The restoration of agricultural fields to saline wetlands may result in an increase in net MeHg production.

5.1.1 Redox Status

The agricultural land that is used for coastal restoration generally has a low elevation and the soil structure has low permeability due to deflocculation and compaction (Tempest *et al*. 2014, Crooks *et al*. 2002). Following de-embankment, sediment waterlogging and organic matter mineralisation causes oxygen levels to deplete which results in *Eh* decreasing (Macleod *et al*. 1999). A decrease in *Eh* can significantly increase metal mobility and decrease the metal-binding capacity of humic materials (Du Laing *et al*. 2009) and therefore, changes in sediment chemistry such as *Eh* can alter the partitioning, and hence mobility and bioavailability, of contaminants (Macleod *et al*. 1999). Inorganic Hg[II] is most susceptible to methylation when it is in the dissolved phase and least susceptible when it is in the particulate state (Davis *et al*. 2003) because the compound is too large to pass over the cell membrane. See Chapter 2, Section 2.5 for full details.

The sediment-water interface is often the main site for mercury methylation and can be a source of MeHg to the overlying water column (Mason *et al*. 1998). Tidal systems experience daily cycling water levels. High tide produces waterlogged and anoxic sediments creating the appropriate conditions for Hg methylation. At low tide, sediments are able to drain and *Eh* can increase which facilitates the oxidation of any by–products produced by anaerobic carbon mineralisation (i.e. sulphide). Subsequently oscillating *Eh* can recycle reduced sulphur pools by oxidation to sulphate, promoting SRB activity and Hg methylation (Hall *et al*. 2008).

5.1.2 Salinity

Coastal wetlands can provide conditions favourable to SRB that facilitate Hg methylation (Marvin-DiPasquale and Agee 2003, Benoit *et al*. 1998, Hall *et al*. 2008). In saline environments, sulphate concentration, a major component of seawater, is higher than freshwater environments. Increasing sulphate concentrations has been shown to increase Hg methylation by stimulating the activity of SRB. However, Hg methylation has generally been found to be lower in estuarine sediments compared to freshwater sediments because high sulphide levels inhibit Hg methylation rates by either creating complexes that are too large to diffuse over the SRB membrane or by removing inorganic Hg from the solution and precipitating it into the solid phase as HgS (Benoit *et al*. 2003). High sulphide levels make $HgHS_2^-$ likely to be the major Hg-S complex which is less bioavailable than HgS^0 to methylating bacteria. Maximum Hg methylation rates will be observed in sediment with neutral Hg complexes such as HgS^0, and will be less evident in highly reducing sediments where sulphide concentrations will result in larger negative complexes, such as HgS_2^- and $Hg(S_x)_2^{2-}$ becoming more prevalent (Fitzgerald *et al*. 2007). Also, as sulphate levels increase, and consequently sulphide (a by-product of SRB metabolic activity) levels may also increase, MeHg reacts with H_2S to produce volatile dimethylmercury (Benoit *et al*. 1999) which can diffuse to the atmosphere.

Salinity also affects Hg-DOM binding because other ions, such as chloride, sulphate, and hydroxide, compete with DOM to form metal-ligand complexes, especially in oxic waters. Mercury-chloride complexes are thought to be important in high chloride oxic conditions (e.g., seawater) they form negatively-charged species and inhibit uptake by SRB (Barkay *et al*. 1997, Davis *et al*. 2003); the Hg[II] ion exists primarily as $HgCl_4^{2-}$and $HgCl_3^-$ (Ullrich *et al*. 2001). $HgCl_2$ is not thought to be present in high chloride concentrations (Mason *et al*. 1995). Barkay *et al*. (1997) showed that availability of Hg[II] is reduced in estuarine and marine environments compared to that in freshwater systems and attributed this to an increased proportion of negatively charged $HgCl_3^2$ and $HgCl_4$.

5.1.3 Interaction of redox potential and salinity concentrations

Alternating oxic/anoxic conditions can recycle sulphide to sulphate which in turn releases Hg[II] into porewater. High sulphate concentrations have been shown, through numerical modelling and laboratory experiments, to only inhibit Hg methylation in soils with low *Eh* due to this build-up of sulphide; sulphate does not inhibit the methylation of Hg in slightly reduced environments (i.e. oxic/anoxic boundary; Benoit *et al*. 1999). In slightly reduced soils or at the oxic-anoxic interface, sulphide is quickly oxidised to sulphate or removed from the porewaters through diffusion, therefore stopping the build-up of this by-product and the production of larger negative complexes (HgS_2^- and $Hg(S_x)_2^{2-}$).

5.1.4 Aims and Objectives

Previous chapters (Chapter 4) have highlighted the importance of the short-term implications of de-embankment on Hg methylation. Here, results are reported for a laboratory study which examines the short-term effects of salinity and redox on methylmercury concentrations in agricultural soil.

The objective for this study (Objective 5) was to examine the sediment MeHg concentrations in terrestrial soils incubated under different combinations of *Eh* (oxic, anoxic and fluctuating) with water containing sulphate concentrations comparable to seawater and water containing no sulphate, over a period of eight weeks. Specifically, the timing and magnitude of MeHg production in terrestrial soils incubated under various *Eh* conditions, with water containing sulphate concentrations, was tested.

5.2 Methodology

A plug method (Aller and Mackin 1989) was used to test the effects of *Eh* and salinity on MeHg production in agricultural soil. For full details see Section 3.2.1. A summary of the analytical methods used is shown in Table 5.1.

Table 5.1
Summary of methodology for Chapter 5

Sample	Number of samples	Replicates	Subsampled?	Total number of samples
Sediment plugs	72	2	Composited	144

Analyte of interest	Method of quantification	Full method description
Sediment THg	AAS	Section 3.3.1
Sediment MeHg	CV-AFS	Section 3.3.2
Carbon	Elemental analyser	Section 3.4.6
Nitrogen	Elemental analyser	Section 3.4.6

5.2.1 Laboratory Analysis

THg was measured following US EPA method 7473 (USEPA 1998). High quality data was assured with sample blanks, sample duplicates and standard reference material (MESS-3, a marine sediment reference material for trace metals certified by National Research Council Canada). Precision, measured as relative standard deviation (% RSD), was 6.8 % (n = 18). Blank samples were below detection and the mean recovery of THg in MESS-3 was 108 % (101 – 123 %, n = 12).

Sediment MeHg was measured by CVAFS (Bloom 1989) following US EPA method 1631 (USEPA 2002). Quality control included measuring sample blanks, duplicate samples, spiked samples, and a sediment reference material (ERM-CC580, an estuarine sediment). Precision (% RSD) was 13 % (n = 12). The mean recovery of MeHg in ERM-

CC580 was 80 % (74 – 92 %, *n* = 24) and spike recoveries averaged 116 % (99 – 127 %, *n* = 24).

The sediment samples were also analysed for carbon and nitrogen content. Quality control included measuring sample blanks, triplicate samples and a standard every 10-15 samples to check for instrument drift. Precision (% RSD) was 15 % (*n* = 12) for nitrogen and 4.9 % (*n* = 12) for carbon. Blank samples were below detection.

THg and MeHg in the overlying water body were not quantified because a) the values were not necessary to answer Objective 5 and b) too much water would have had to be removed from the tanks in order to obtain a reliable concentration.

5.2.2 Statistical analysis

First, a Spearman's rank correlation analyses were used to test association between THg, MeHg, % MeHg, and total carbon and nitrogen concentrations. Data that were not normally distributed (% MeHg and nitrogen) were successfully transformed using 1/sqrt(Y). A two-way repeated-measures ANOVA was used to test for significant differences between all treatments for THg, and total carbon and nitrogen concentrations. Where the assumption of sphericity is violated, the degree of freedom for the effect is adjusted and the corrected F-value is used. The p*ost hoc* test, Bonferroni, was carried out where the two-way repeated-measures ANOVA analysis gave a significant result.

A non-linear regression analysis was then carried out on MeHg data, fitting a modified Ricker function to the untransformed data. The Ricker function is a standard curve for hump-shaped ecological patterns that are positively skewed (Ricker 1973, Laws and Archie 1981). A Ricker function can be defined as:

$$y = axe^{-bx} \qquad Eq(1)$$

where *y* is the dependant variable (MeHg concentration in this example); *x* is the independent variable (time in days); *a* is a fitted parameter that describes the initial rate of increase in *y* for small values of *x*; *b* is a fitted parameter that describes the subsequent non-linear decrease in *y* for higher values of *x*; and *e* is the base of the natural logarithm. The standard Ricker function was modified so as to include a constant, *c*:

$$y = axe^{-bx} + c \qquad Eq(2)$$

The constant *c* prevents *y* declining to zero for high values of *x*, and can be interpreted as background MeHg concentration. The size and duration of the pulse of MeHg (the cumulative excess exposure) was quantified for all treatments by integrating the equation above the background concentration.

The net production (i.e. production minus consumption) is given by differentiating equation 2 with respect to *x* (Equation 3). Positive values of the derivative indicate a positive slope in the time series and therefore net production; negative values of the derivative indicate net consumption.

$$\text{Rate of change} = a\,e^{-bx}(1-bx) \qquad Eq(3)$$

Furthermore, equation 2 can be rearranged as follows to give the peak MeHg concentration as predicted by the model;

$$\text{Peak MeHg} = \frac{a}{b}e^{-1} + c \qquad Eq(4)$$

Finally, the time at which peak MeHg concentration occurs (in days) is given by equation 5.

$$\text{Peak time} = \frac{1}{b} \qquad Eq(5)$$

Initially, each of the three parameters was estimated separately for each redox-salinity combination. Then, for each parameter, the number of predictor variables was reduced systematically (redox-salinity combination, redox only, salinity only, constant across all treatments) to find the most parsimonious model. Model selection was based on AICc, the Akaike Information Criterion corrected for small sample size (Burnham and Anderson, 2002). The procedure for fitting the model to data is given in Appendix 3. Statistical analyses were performed using SPSS 19.0 and R 3.0.1 (R Development Core Team 2013), and package nlme (Pinheiro *et al*. 2014) and AICcmodavg (Mazerolle 2015).

5.3 Results

5.3.1 THg Concentrations

In general, THg sediment concentrations in the soil were low, ranging from 56 to 72 ng g^{-1} (mean: 63 ng g^{-1} dry weight). The amount of THg stayed constant throughout the experiment (within measurement error) with no clear changes over time (Figure 5.1), except in the anoxic-saline water treatment where THg decreased after 7 days. There were significant differences between DI water and saline water treatments with the saline treatment containing significantly less THg than the DI water treatment (Table 5.2). There were no significant differences in THg between the three levels, oxic vs anoxic vs fluctuating, and no significant interaction between *Eh* and salinity.

Table 5.2
Repeated measures two-way ANOVA results

Factors	*df*	MS	F-statisitc	*p*-value
THg				
Redox	2	13.0	0.895	0.416
Salinity	1	277	38.7	<0.001
Interaction	2	21.0	1.47	0.241
Total Carbon				
Redox	2	0.002	0.088	0.916
Salinity	1	0.319	18.8	<0.001
Interaction	2	0.030	1.24	0.300

5.3.2 MeHg Concentration and % MeHg

MeHg sediment concentrations ranged from 56 to 287 pg g^{-1} (mean: 128 pg g^{-1} dry weight). Over the eight week experiment, MeHg concentrations peaked within the first 10 days for all treatments (Figure 5.2) with saline treatments containing noticeably larger peaks than DI water treatments, as well as anoxic and fluctuating treatments containing larger peaks than oxic treatments.

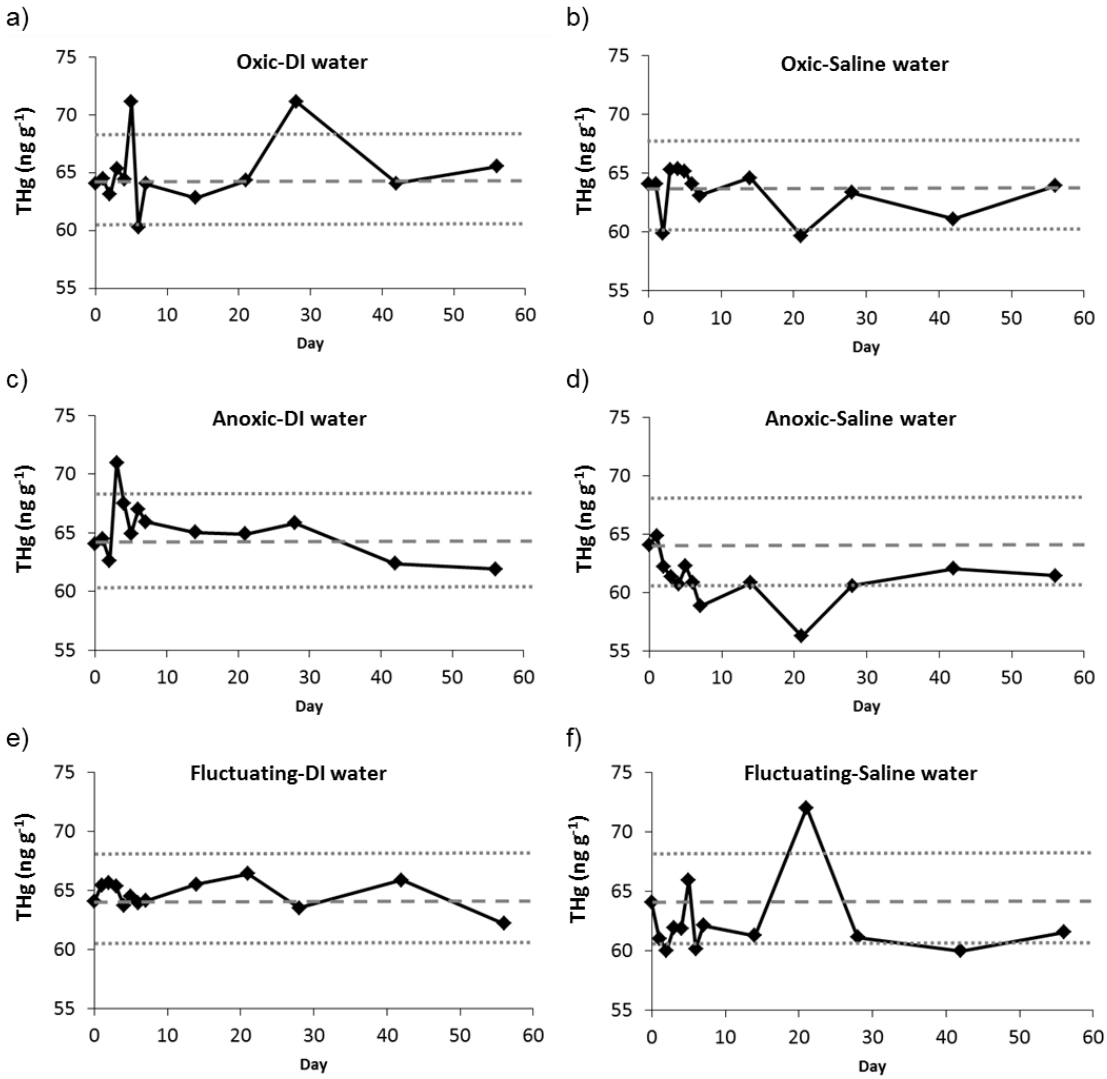

Figure 5.1. Total Hg sediment concentrations in treatments (a) oxic-DI water, (b) oxic-saline water, (c) anoxic-DI water, (d) anoxic-saline water, (e) fluctuating-DI water, and (f) fluctuating-saline water conditions over a period of 56 days. Dotted line represents starting concentration +/- RSD.

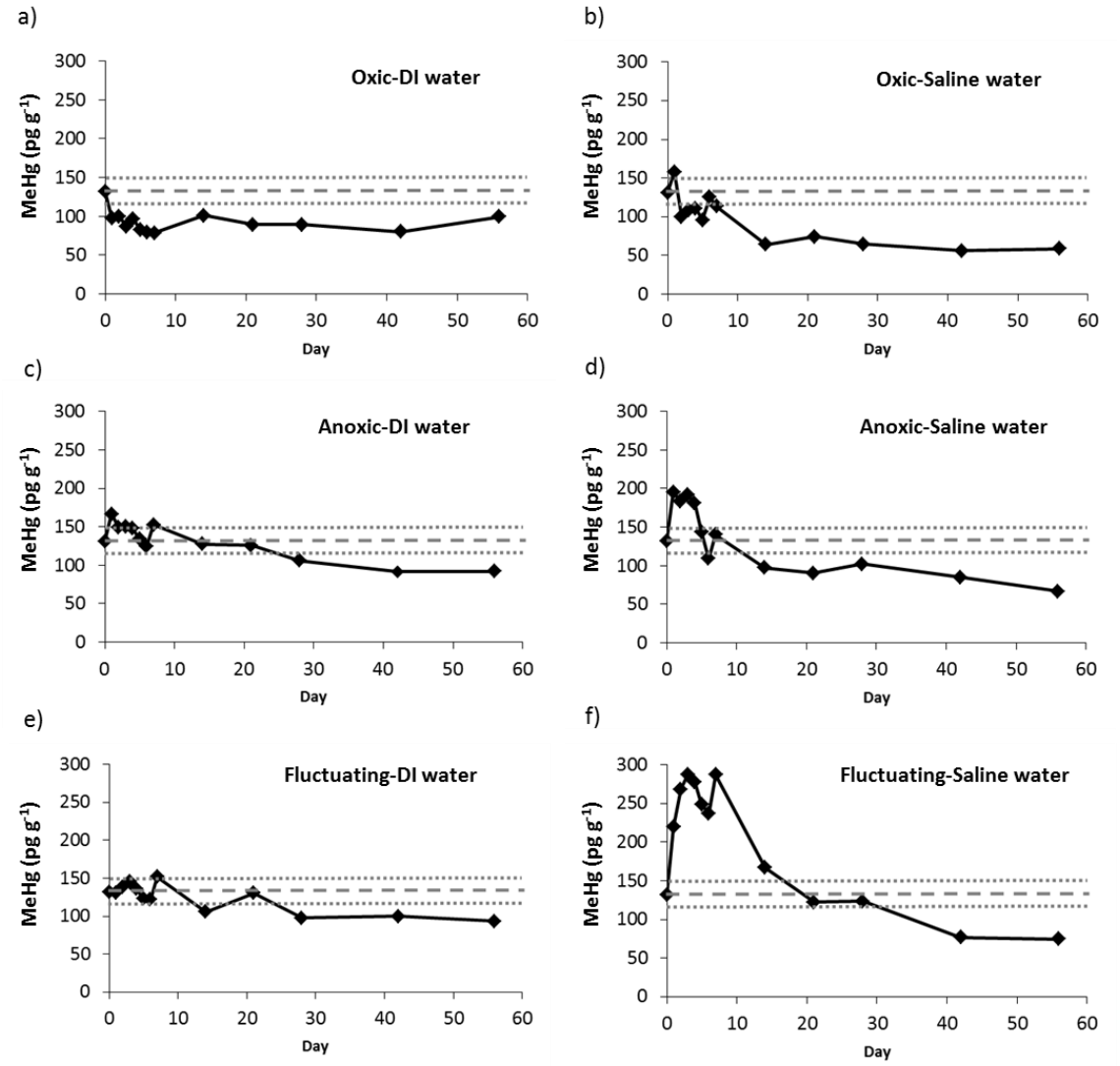

Figure 5.2. Methylmercury sediment concentrations in (a) oxic-DI water, (b) oxic-saline water, (c) anoxic-DI water, (d) anoxic-saline water, (e) fluctuating-DI water, and (f) fluctuating-saline water conditions over a period of 56 days. Dashed line represents initial value. Dotted line represents starting concentration +/- RSD.

The change in MeHg concentration over time with both observed and modelled data is shown in Figure 5.3. The modified Ricker function gave an acceptable fit to observed data (Figure 5.4) except for the oxic DI-water and oxic saline-water treatments where the model failed to give significant estimation of the initial value (i.e. parameter *a*) as well as the control on the rate of decrease in MeHg concentration for oxic treatments (i.e. parameter *b*). Values for the parameters across the different treatments and their statistical significance are given in Table 5.3.

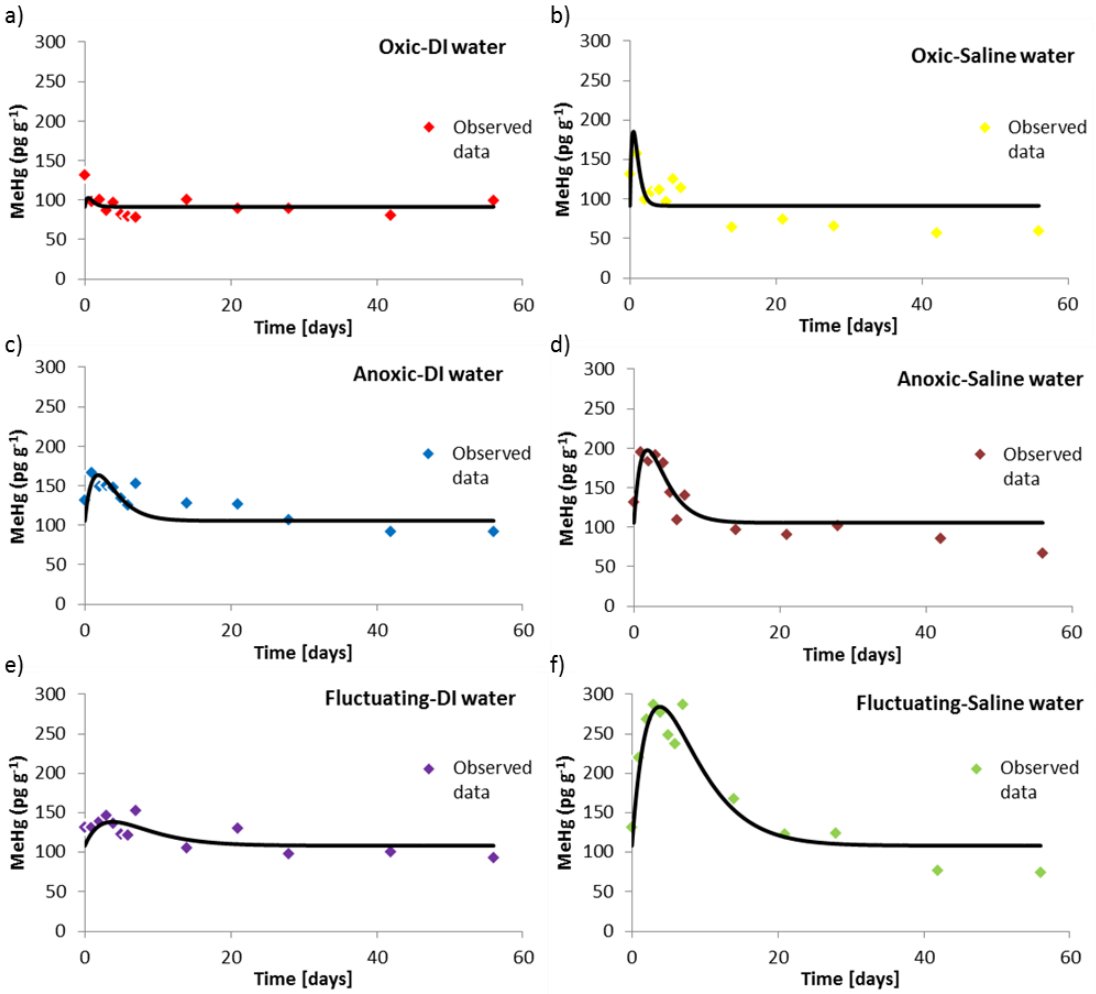

Figure 5.3 Change in MeHg concentration over time (pg g^{-1}), with model fit

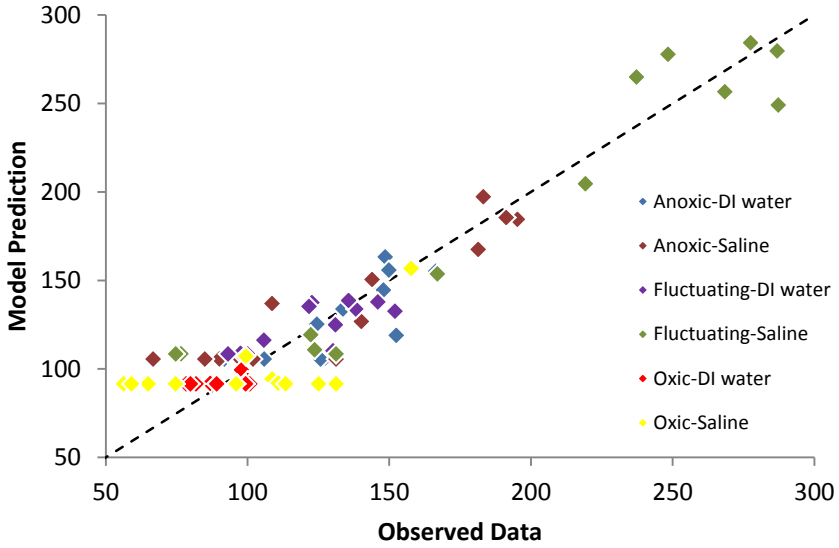

Figure 5.4 Predicted model values compared to observed data. Dashed line represents 1:1 relationship; points above the line indicate model overestimation; points below the line indicate underestimation.

Table 5.3
Summary of modified Ricker's function fitted to observed data. Akaike's Information Criterion (AIC) was 703.76 (78 degrees of freedom)

Parameter	Treatment	Value	Std. Error	*p*-value
a	Anoxic - DI water	85.5	23.8	< 0.001
a	Anoxic - Saline water	136	30.8	< 0.001
a	Fluctuating - DI water	21.5	8.14	< 0.05
a	Fluctuating - Saline water	125	14.4	< 0.001
a	Oxic - DI water	65.5	186	0.726
a	Oxic - Saline water	541	741	0.468
b	Anoxic	0.540	0.070	< 0.001
b	Fluctuating	0.260	0.020	< 0.001
b	Oxic	2.12	1.28	0.103
c	Anoxic	106	5.71	< 0.001
c	Fluctuating	108	6.21	< 0.001
c	Oxic	91.4	4.39	< 0.001

Parameter *a* gives an initial rate of net MeHg concentration (Table 5.4). However, the oxic treatments for parameter *a* did not give a significant value and so these values are excluded from interpretation. The saline treatment for both anoxic and fluctuating conditions contained higher initial net rate of MeHg production.

Table 5.4
Derived model outputs

Treatment	Initial rate of net MeHg production (pg g^{-1} day^{-1})	Peak Concentration (pg g^{-1})	Cumulative exposure over 56 days (pg g^{-1})
Anoxic - DI water	85.5	163	290
Anoxic - Saline water	136	198	461
Fluctuating - DI water	21.5	139	315
Fluctuating - Saline water	125	284	1833
Oxic - DI water	65.5	103	14.6
Oxic - Saline water	541	186	121

The timing of the pulse of MeHg varied between treatments (Table 5.2). The peak in MeHg concentration occurred at 0.47 days for oxic, 1.84 days for anoxic treatments and 3.83 days for the fluctuating treatments. This is reflected in the smaller *b* parameter value for fluctuating treatments which results in the peak concentration being reached more slowly and the decline occurring less rapidly. The peak concentration and cumulative exposure were also larger for saline treatments compared to DI-water treatments. The fluctuating saline treatment contained the highest concentrations, and then the anoxic treatment, with the oxic treatment containing lowest MeHg concentrations.

Net production of MeHg, as given by the rate of change in the model, is given in Eq. 5.3 and outputs are shown in Figure 5.5. The DI water treatments start with lower net MeHg production rates than saline treatments, although the oxic-saline treatment also decreases quickly to a negative value, indicating net consumption of MeHg. The fluctuating-saline treatment starts with a high net MeHg production rate and

decreases more slowly to negative values before levelling out at zero net MeHg production, indicating that net production equals net consumption.

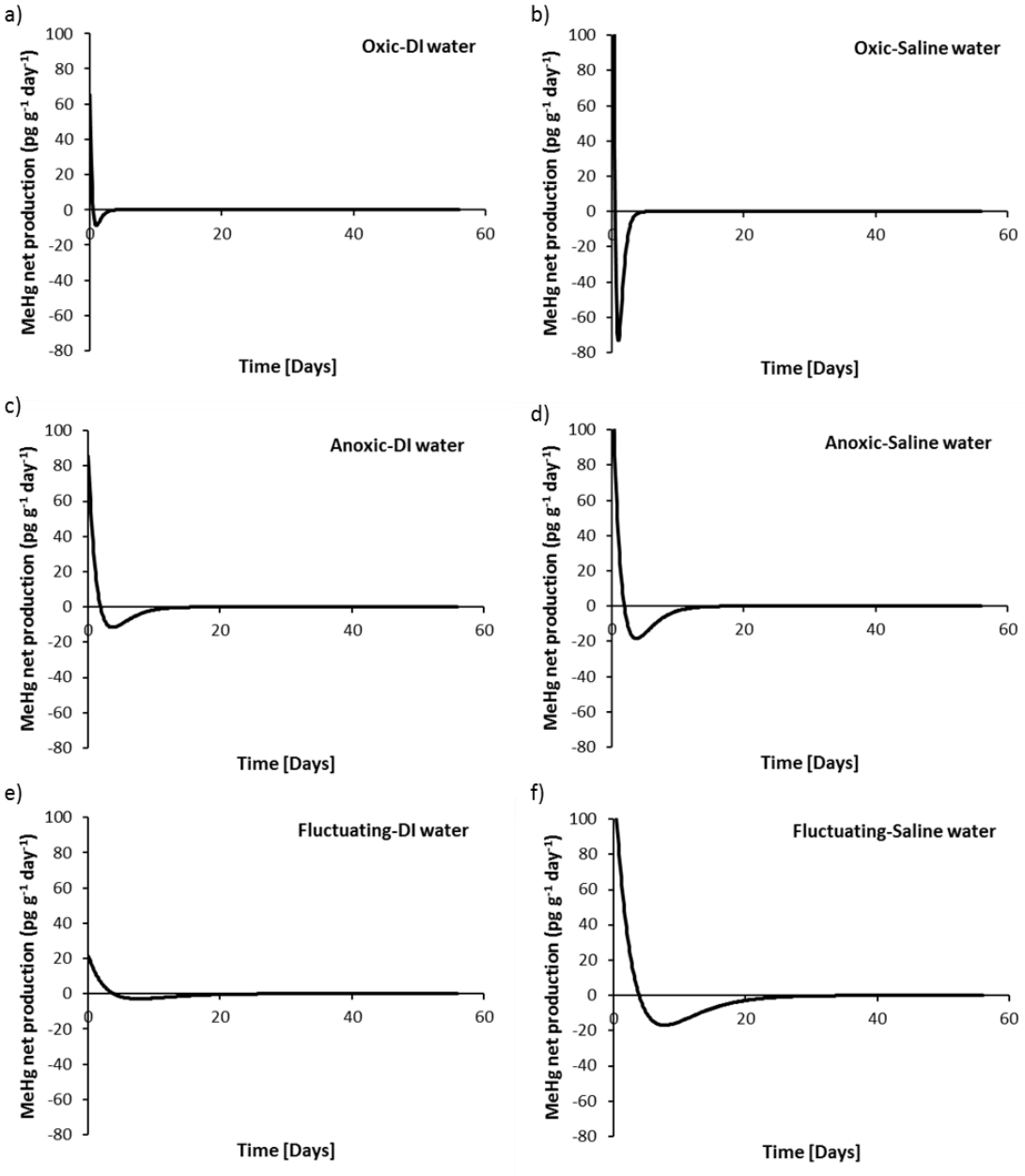

Figure 5.5 Net production of MeHg as given by the rate of change in the model given in equation 3.

The proportion of THg occurring as MeHg (% MeHg) was used to estimate the Hg methylation potential in these agricultural soils. MeHg concentrations ranged from 0.07 % to 0.48 %. Over the eight week period, concentrations showed similar variation to MeHg concentrations (Figure 5.6) with fluctuating saline treatments containing higher peaks than oxic DI-water treatments.

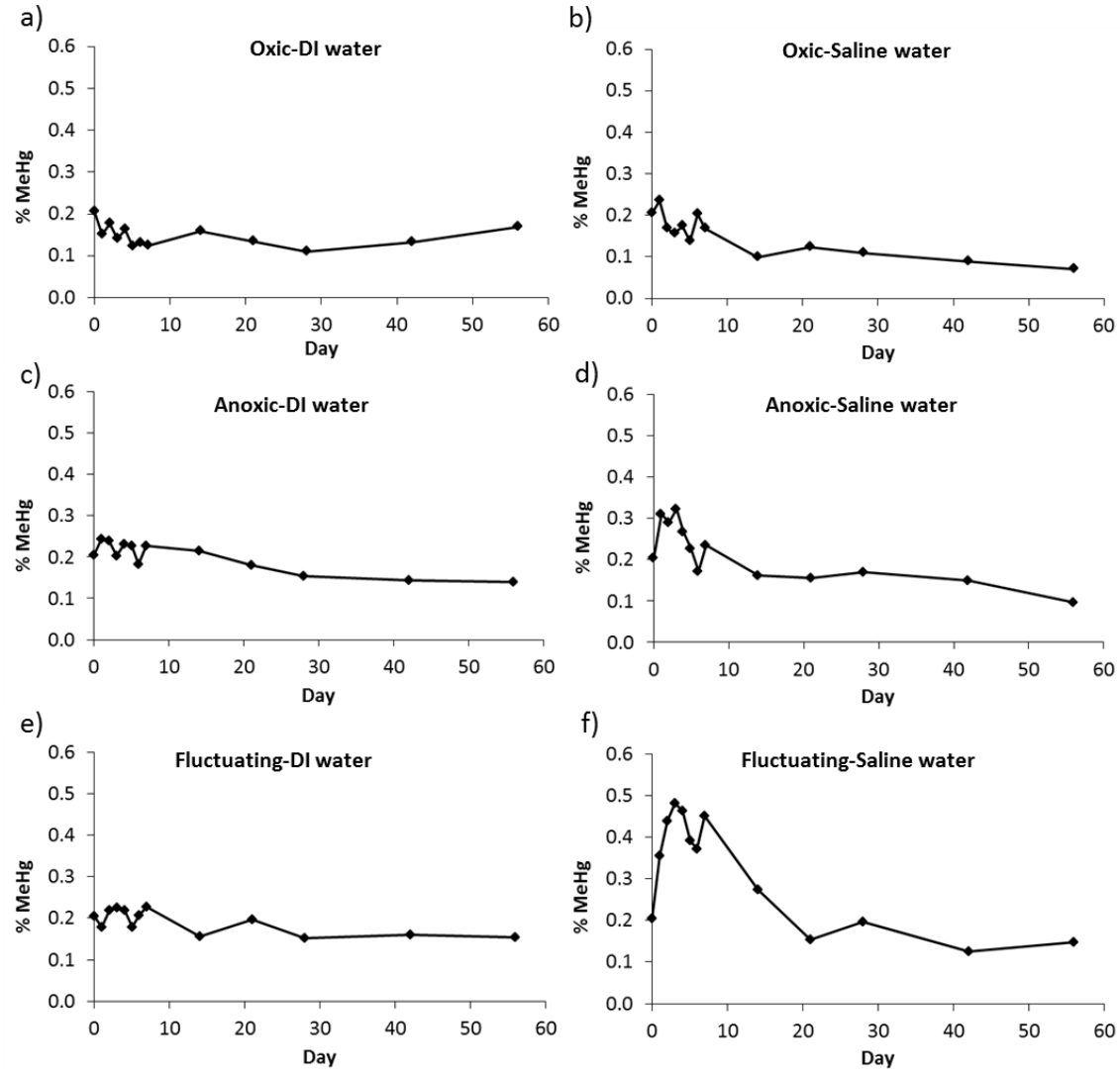

Figure 5.6 % MeHg in (a) oxic-DI water, (b) oxic-saline water, (c) anoxic-DI water, (d) anoxic-saline water, (e) fluctuating-DI water, and (f) fluctuating-saline water conditions over a period of 56 days.

5.3.3 Total Carbon

Total carbon (% TC) concentrations ranged from 1.7 % to 2.3 %, averaging 1.95 % for all sample treatments. The amount of %TC stayed fairly constant throughout the experiment (within measurement error) with no clear changes over time (Figure 5.7). There were significant differences between DI water and saline water treatments with DI water treatments containing more % TC than saline water treatments (Table 5.2). The *Eh* treatment had no significant effect on % TC and there was no significant interaction between *Eh* and salinity. A correlation matrix between Hg variables and %TC is shown in Table 5.5. Total carbon has a positive significant correlation with THg (0.258, $p<0.05$) and no correlation with MeHg or %MeHg.

Table 5.5
Spearman's rank correlation matrix. Significant values are shown in bold.

		% MeHg	MeHg (pg/g)	THg (ng/g)	% TC
% MeHg	Spearman's Rank	**1**	**0.969**	-0.1	0.054
	Sig.		**0.000**	0.583	0.650
MeHg (pg/g)	Spearman's Rank		**1**	0.056	0.070
	Sig.			0.640	0.558
THg (ng/g)	Spearman's Rank			**1**	**0.258**
	Sig.				**0.029**
% TC	Spearman's Rank				**1**
	Sig.				

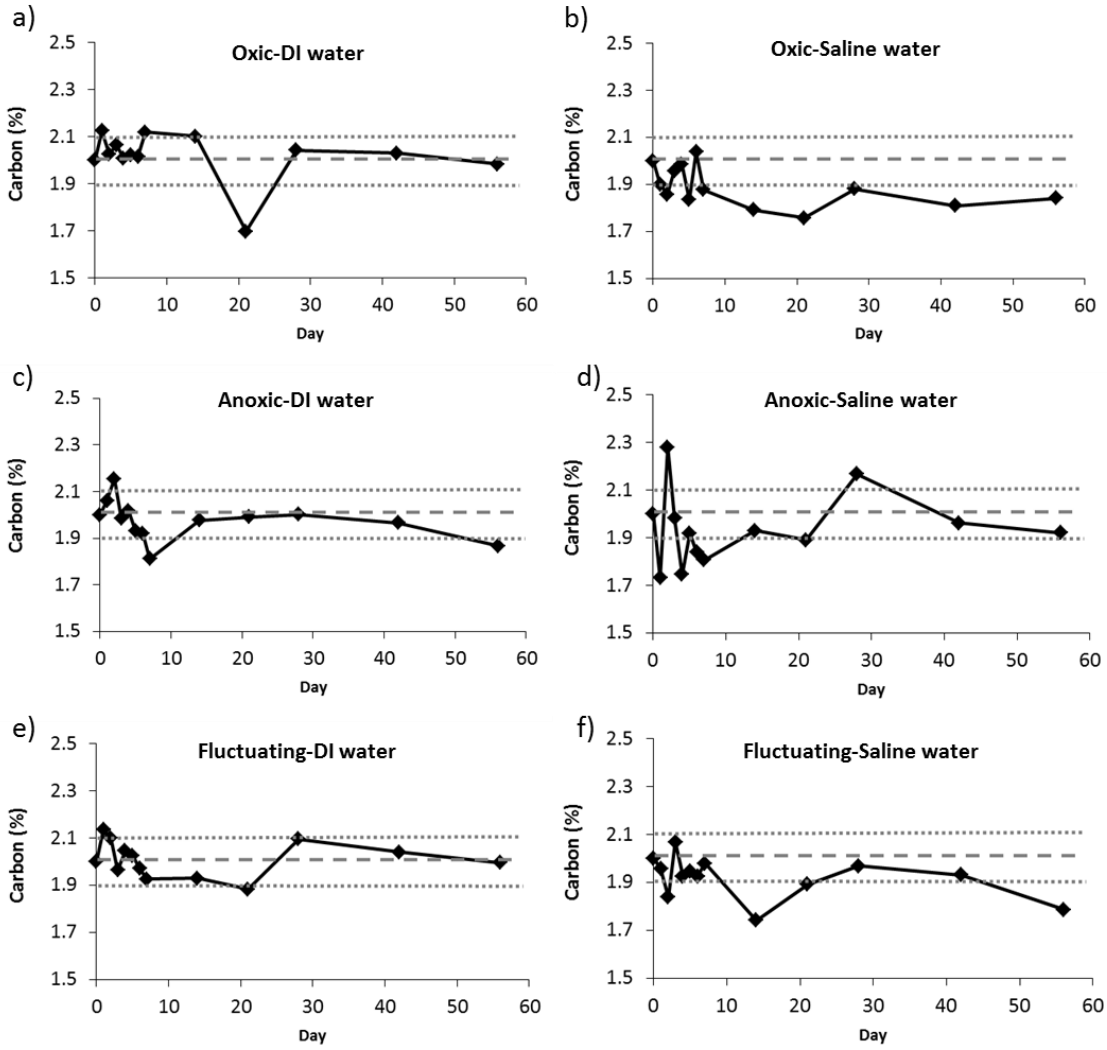

Figure 5.7 Total carbon (%) in (a) oxic-fresh, (b) oxic-saline, (c) anoxic-fresh, (d) anoxic-saline, (e) fluctuating-fresh, and (f) fluctuating-saline conditions over a period of 56 days. Dotted line represents starting concentration +/- RSD.

5.3.4 Total Nitrogen

Total nitrogen (% TN) was quantified for all treatments and ranged from 0.09 % to 0.49 %, the mean for all treatments being 0.21 % ± 0.01. Total nitrogen concentrations were at method detection limits for the analytical method. Therefore, statistical analysis is not discussed for this parameter.

5.4 Discussion

MeHg production occurred under both anoxic and fluctuating conditions but was limited (although not non-existent) under permanently oxic conditions. MeHg production was higher under fluctuating *Eh* conditions than under permanently anoxic conditions and MeHg production was higher in soils incubated in saline water than in DI water. There was a peak in MeHg concentration in all treatments (except the oxic-DI water treatment) which resulted from MeHg production being higher than losses of MeHg (i.e. transport to overlying water column or demethylation). The rate of MeHg production then slows down, likely due to the exhaustion of labile carbon as an energy source for methylating bacteria. MeHg concentrations then evens out, however at levels lower than starting values. This is likely due to MeHg content already present in the soil being transported to the overlying water column when the soil was flooded with either saline or DI water.

5.4.1 Influence of redox on mercury biogeochemistry

The oxic treatments produce lower peak MeHg concentrations and contain lower cumulative exposure of MeHg over 56 days than both anoxic and fluctuating treatments. In this experiment, the oxic soils do not contain the *Eh* conditions required for MeHg production. SRB (known methylators) thrive in anoxic sediments (Hall *et al*. 2008) and therefore a decrease in *Eh* is favourable for Hg methylation and therefore greater MeHg peaks are found within the anoxic treatment. The fluctuating treatments contain the highest peaks in MeHg concentration, likely because the fluctuating *Eh* conditions allow the re-oxidation of reduced sulphides. Sulphide, a by-product of SRB metabolic activity, has been shown to inhibit Hg methylation by either creating Hg complexes that are too large to diffuse over the SRB membrane or by removing inorganic Hg from the solution and precipitating it into the solid phase as HgS (Benoit *et al*. 2003)**.** A decrease in *Eh* is favourable for metal sulphide precipitation because the reduction of sulphate to sulphide promotes the formation of insoluble metal sulphides which reduces the availability of Hg as well as other trace metals in the soil (Teuchies *et al*. 2012, Teuchies *et al*. 2008).

Over time, a small increase in MeHg concentration is apparent in the oxic-saline treatment within the first few days suggesting that MeHg production is greater than both the partitioning of MeHg into water and demethylation. Although the overlying water is oxic, the plugs may still remain anoxic. This will depend on whether there is exchange of free oxygen from the overlying water column with the sediment plug. In submergent environments chemical and biological oxygen demands can exhaust the dissolved oxygen within a few millimetres of the sediment surface (Compeau and Bartha 1987) and therefore micro-anoxic environments form allowing SRB to become active to some extent. A small peak is evident in the oxic-saline treatment. A possible explanation for this could be that micro-anoxic pockets are forming within the sediment allowing SRB to become active. The fluctuating-saline treatment contained the highest peaks which occurred after 3.8 days. The fluctuating treatments allowed for the re-oxidation of sulphide. It is likely that after 3.8 days, the labile carbon source had diminished because there was no other external source of organic carbon to these soils. In natural conditions, organic matter would be delivered to the sediment during tidal inundation from the overlying water column and from plant roots (Windham-Myers *et al*. 2009) and so would not be a limiting factor for Hg methylation.

Organic matter content can limit Hg methylation because Hg complexation with organic matter (either solid phase organic matter or DOC) creates molecules that are too large to cross the cell membranes on the SRB (Ravichandran 2004, Choi and Bartha 1994, Barkay *et al*. 1997). Here, THg significantly correlates with % TC data suggesting that organic matter content has a control on the distribution of THg. This was also found in Chapter 4. There was no significant correlation between % TC and MeHg. However, the % TC present may not correlate strongly with MeHg concentrations if it is of poor quality and not available to SRB. Measuring % TC does not give an indication of the quality or lability of the organic matter.

Eh had no significant effect on THg concentration in the soil plugs. Although reducing conditions may have caused a release of THg into solution, THg in soil is generally measured in ppb, whereas porewater THg is measured in ppt and therefore the magnitude of change would not be large enough to be able to quantify a significant difference.

The results indicate that MeHg production is also occurring in DI water treatments and therefore SRB activity is not likely to be the main source. However, inundation can also increase Hg methylation by stimulating iron reducing bacteria (FeRB) which have also been shown to methylate Hg (Mitchell and Gilmour 2008). Inundation and reducing conditions can mobilise Fe and MacLeod *et al*. (1999) showed that sediment Fe concentrations decreased 5 fold after inundation. This is attributed to the dissolution of Fe oxides and the reduction of Fe^{3+} to Fe^{2+}. The reduction of Mn[IV] and Fe [III] to Mn[II] and Fe [II] respectively is associated with the increase solubility of Fe and Mn as well as other metals, such as Hg, bound to the surface of the oxides (Otero *et al*. 2009, Portnoy and Giblin 1997). As the sediment becomes reduced, as a consequence of microbial degradation of organic matter, Fe is reduced and released from the Fe oxides, leaving any Hg associated with Fe oxides in solution (Heyes *et al*. 2004, Choe *et al*. 2004). THg in solution is more bioavailable for methylation than particulate THg (Gilmour and Henry 1991, Ullrich *et al*. 2001). FeRB is probably the main pathway of MeHg production in these sediments, although higher concentrations are found in saline conditions and therefore SRB are more efficient and produce more MeHg than FeRB.

5.4.2 Influence of salinity on methylmercury concentrations

Samples treated with saline water had significantly less THg than DI water treatments, indicating that a portion of THg has been mobilised and released from the sediment phase. Flooding former agricultural land with saline water has been shown to alter the mobility and availability of trace metals within the soil (Speelmans *et al*. 2007, Teuchies *et al*. 2012). Although flooding soils, independent of salinity, can increase

metal availability, here, THg in the DI water treatment contained THg concentrations within the % RPD of the initial sample (measured before samples were flooded). Therefore the differences seen are due to salinity rather than the act of flooding the soils.

Changes in salinity can alter the partitioning of metals between solid and aqueous phases through two main processes. Firstly, high Cl^- concentrations reduce the amount of Hg associated with the particulate phase, most likely due to competition of Cl^- for binding sites (eg. Cl^- and SO_4^{2-}; Ullrich *et al*. 2001, Morel *et al.* 1998). In the presence of increasing chloride concentrations complexes of Hg[II]-polychlorides prevail, e.g., $HgCl_3^-$ and $HgCl_4^{2-}$ (Fitzgerald *et al*. 2007, Liu *et al*. 2012). Secondly,an increase in salinity is associated with an increase in major cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) that compete with heavy metals for the sorption sites (Du Laing *et al*. 2009). Therefore, inundation with saline water can cause the release of trace metals into the aqueous phase due to cation exchange (Speelmans *et al*. 2007). This increase in ion exchange may have resulted in the mobilisation and partitioning of THg from the solid phase into the porewater in the saline treatment samples.

MeHg peaks are significantly larger within the first 10 days in saline treatments before decreasing to levels similar to DI water treatment levels. MeHg concentrations are also significantly higher in saline samples compared to DI water samples (Figure 5.2). There are two possible explanations for these differences.

Firstly, samples that have been inundated with saline water have significantly lower sediment THg concentrations than fresh water samples (65 ng g^{-1} in saline treatment compared with 61 ng g^{-1} in DI water treatments), suggesting that THg has been mobilised into solution and is therefore more bioavailable for methylation than particulate THg (Gilmour and Henry 1991, Ullrich *et al*. 2001, Davis *et al*. 2003). Although some of this THg will be lost to the overlying water column, approximately

only 1 % of THg is methylated (Ullrich *et al*. 2001) and so only a small proportion needs to be available in pore water for methylation to occur.

Secondly, in the overlying saline water there is abundant SO_4^{2-} which may stimulate SRB (Fitzgerald *et al*. 2007). Carbon mineralisation rates have been shown to significantly increase following saltwater intrusion (Weston *et al*. 2011) due to the increase in activity of SRB and SRB are responsible for 50-90 % of the organic carbon mineralisation in coastal sediments (Fitzgerald *et al*. 2007). The majority of MeHg production in coastal wetlands is produced by in situ methylation of Hg[II] by sulphate-reducing bacteria (SRB) (Fitzgerald *et al*. 2007) and therefore, an increase in SRB activity will result in an increase in MeHg concentration. Total carbon concentrations also decreased in saline samples supporting that carbon mineralisation, due to SRB activity, is greater in saline treatments (Figure 5.7). There is no significant correlation however, between MeHg and carbon concentration although, a significant correlation would not be expected in DI water treatments because carbon availability is not the limiting factor for Hg methylation. When DI water samples are removed from data analysis, a significant positive correlation is found between MeHg and carbon concentration (r = 0.335, p<0.05). The extent of Hg methylation is also dependent on the quality of organic carbon (Kelly *et al*. 1997) and therefore in environments with more labile carbon, for example agricultural sites that are vegetated at the time of tidal inundation (Windham-Myers *et al*. 2009), the methylation rates could be much higher. These samples were collected from bare agricultural fields and so % TC levels are relatively low.

5.4.3 Implications for coastal restoration

Highest concentrations of MeHg were measured in the fluctuating treatment where the formation of metal sulphides will be limited due to the oxidation of reduced species during the oxic cycle (i.e. re-oxidation of sulphide to sulphate). This indicates that in recently restored coastal wetlands, where the sediment is waterlogged and anoxic (Morris *et al*. 2014), Hg methylation will occur but will be limited. As the

restored saltmarsh develops and accumulates sediment, the elevation increases and the sediment can drain and fluctuating *Eh* conditions are created during the tidal cycle. MeHg is produced at high tide when the sediment is waterlogged and anoxic. During low tide, sulphide is re-oxidised to sulphate and does not form insoluble metal sulphides allowing for much higher MeHg concentrations within the sediment (Morris *et al*. 2014). Furthermore, the cumulative exposure of MeHg over 56 days for fluctuating saline treatments was an order of magnitude bigger than oxic saline treatments and two orders of magnitude bigger than oxic DI water treatments. These finding could have significant implications for future tidal inundation schemes, especially sites in contaminated areas with higher THg concentrations. Although the site may not be a large source of MeHg within the first few weeks, the site could produce higher concentrations of MeHg as the sites elevation increases and the site drains during tidal cycles.

5.5. Conclusions

Flooding agricultural soils with saline water has been shown to significantly increase sediment MeHg concentrations. Peaks in MeHg concentration were evident in anoxic samples. However, significantly larger peaks were evident in samples taken from the fluctuating (oxic/anoxic) conditions. This is an important finding for coastal managers because it indicates that peaks of MeHg could be produced immediately following tidal inundation and that MeHg could potentially increase over time as the site develops to a tidal regime more comparable to a natural saltmarsh. This supports the findings from Chapter 4 (Morris *et al*. 2014), where MeHg concentrations were highest in the oldest restored site.

THg concentrations in sediment decreased in saline treatments indicating that cation exchange may have resulted in the increased mobility changes of THg and released to porewater and/or the overlying water column in saline environments. MeHg concentrations increased in saline samples which was attributed to increased SRB activity in saline samples as well as the increased bioavailability of Hg(II) for methylation by SRB. Peaks in MeHg concentrations in fresh water treatments are attributed to micro-anoxic environments forming within the sediment.

MeHg in these soils is likely produced by SRB and therefore environmental factors that influence microbial activity need to be considered when trying to understand Hg methylation in restored coastal wetlands. The controls on Hg methylation could vary between sites depending on pH, redox status, Fe concentration and sulphur cycling and need to be fully understood in order to determine the potential ecological impact of coastal restoration on the surrounding environment. Previous studies have indicated that permanently flooded soils produce high MeHg concentration however this is the first study to show that fluctuating saline conditions could produce a pulse of MeHg, an important consideration for coastal managers.

Chapter 6: Controls on Hg methylation in recently restored coastal saltmarshes, with specific emphasis on the effects of ecosystem restoration on Hg methylation.

6.1 Introduction

De-embankment and saline inundation leads to a series of physical and chemical changes that impacts the biogeochemical cycling within the sediment (see Chapter 4). De-embankment can create waterlogged and anoxic sediment which in turn can promote the development of reducing conditions and increased salinity and pH. Following inundation sediment accumulates and the surface elevation increases within the tidal frame, drainage improves and saltmarsh plants will colonise (Garbutt and Wolters 2008, Morris *et al*. 2014). Therefore, overtime since de-embankment the sediment goes through a series of changes such as increased drainage, anoxic to fluctuating oxic/anoxic conditions, lower salinity and increased organic carbon content (Wolters *et al*. 2005, Morris *et al*. 2014). These changes in sediment conditions can alter the mobility of Hg (Emmerson *et al*. 2000, Speelmans *et al*. 2007, Teuchies *et al*. 2012) and increase Hg methylation (Chapter 5). Restored saltmarshes that contain compacted sediment and limited tidal flushing (Tempest *et al*. 2014, Morris *et al*. 2014) may have lower MeHg concentrations than natural saltmarshes within the upper 30 cm profile due to reasons discussed in chapter 4, however concentrations in the surface sediment (where typically MeHg production is highest) may differ. Given that it is the surface sediment that has the most interaction with the overlying water column and hence the wider ecosystem, it is important to understand any differences in controls on Hg methylation in these surface sediments. The controls on MeHg concentrations may also change with time since de-embankment due to ecosystem

development and subsequent changes in the physical and chemical sediment properties.

MeHg concentrations in sediments are mainly controlled by in situ microbial methylation (Kerin *et al*. 2006), predominantly via SRB and FeRB. These microbes convert a small fraction of available Hg[II] to MeHg. A number of factors affect the rate of MeHg formation by influencing the supply of bioavailable Hg[II] and/or activity of methylating microbes. Previous research has identified several proxy indicators of Hg methylation including sulphide concentration, Fe[II] concentration, Hg[II] concentration, organic matter content and redox potential (Sunderland *et al*. 2006, Benoit *et al*. 2003, Compeau and Bartha 1984).

Saltmarsh sediments contain communities of sulphate-reducing bacteria (SRB); the principal group of organisms responsible for Hg methylation (Benoit *et al*. 2003, Compeau and Bartha 1985), although other reducing bacteria such as Fe reducing bacteria (FeRB) can also be important (Mitchell and Gilmour 2008, Kerin *et al*. 2006). SRB thrive in organic-rich, anaerobic sediments in aquatic environments (Hall *et al*. 2008), especially in saline ecosystems where sulphate is abundant (Andrews *et al*. 2006) and are responsible for 50-90 % of the organic carbon mineralisation in coastal sediments (Fitzgerald *et al*. 2007). Therefore, in anoxic, organic rich and saline environments (i.e. restored saltmarshes) where SRB activity is enhanced, Hg methylation rates are also likely to be higher in these environments. However, sulphide, a by-product of sulphate reduction, has been shown to inhibit Hg methylation rates at sediment concentrations greater than 2.6 mg g^{-1} (Craig and Moreton 1983) because high sulphide levels make $HgHS_2^-$ likely to be the major Hg-S complex which is less bioavailable than HgS^0 to methylating bacteria (Fitzgerald *et al*. 2007). Daily tidal flooding can decrease sulphide concentrations either by removal of sulphide to the overlying water column or oxidation to sulphate (Benoit *et al*. 1999).

Hg in sediments can be associated with Mn and Fe oxyhydroxides which has a major control on its' bioavailability to methylating bacteria (Lillebø *et al*. 2010). It is therefore

also important to look at Fe and Mn when trying to determine the controls on Hg methylation. In the absence of available O_2, microorganisms use other electron acceptors, such as Mn and Fe oxyhydroxides. Therefore, an increase in porewater Fe and Mn concentrations is indicative of a reducing environment. The reduction of Mn[IV] and Fe [III] to Mn[II] and Fe [II] respectively at the oxic-anoxic boundary is associated with the increased solubility of Fe and Mn bound to the surface of the oxides (Otero *et al*. 2009). Inorganic Hg bound to the surface of the oxides is also released into solution. Inorganic Hg[II] is most susceptible to methylation when it is in the dissolved state (Davis *et al*. 2003). Therefore, dissolved-solid phase partitioning reactions are important for regulating Hg bioavailability which in turn will influence Hg methylation rates (Krabbenhoft *et al*. 2005).

Redox potential has a complex control on MeHg concentrations. In a reducing environment, Hg[II] is released to porewater which is more bioavailable to SRB and FeRB. SRB and FeRB also thrive at the geochemical interface between oxic and anoxic conditions. However, as the redox potential decreases, sulphide concentration increases which can inhibit Hg methylation by reducing the bioavailable pool of Hg[II] (Benoit *et al*. 1999).

Environmental factors can be measured to indicate reducing conditions as well as SRB and FeRB activity (Slowey and Brown Jr 2007). Sediment acid volatile sulphur (AVS; FeS) and chromium reduced sulphur (CRS; FeS_2) can be regarded as temporary sedimentary sinks for sulphides and are almost entirely microbiologically produced (Rickard and Morse 2005). Porewater sulphide, AVS and CRS give an indication of sulphate reduction, whereas an increase in reduced porewater Fe (Fe[II]) gives an indication of increased Fe reduction and FeRB activity.

6.1.1 Aims and Objectives

The following chapter outlines the results and discussion in order to answer objectives 6 and 7 set out in Chapter 2, Section 2.8.

Aim: To understand the controls on Hg biogeochemical cycling in restored coastal wetlands, with specific emphasis on the effects of ecosystem restoration on Hg methylation.

Objective 6: To explore the association between MeHg concentrations and environmental variables known to control Hg methylation in natural and restored coastal wetlands.

Objective 7: To explore how controls on Hg methylation change with time since de-embankment.

6.1.2 Methodology

For full sampling strategy see Methodology, Section 3.2.1. Detailed methods for analysis, sample collection and porewater extraction are given in sections 3.3 to 3.5. A summary of the analytical methods used is shown in Table 6.1.

Table 6.1
Summary of methodology for Chapter 6

Sample	Number of samples	Replicates	Subsampled?	Total number of samples
Surface sediments	60	3	Composited	180

Analyte of interest	Method of quantification	Full method description
Sediment THg	AAS	Section 3.3.1
Sediment MeHg	Alkaline extraction, CV-AFS	Section 3.3.2
pH	pH meter	Section 3.3.3
LOI	Ignition at 450° C	Section 3.3.4
Geochemical (metals)	Aqua regia digestion; ICP-OES	Section 3.3.5
AVS	Sulphide-selective electrode	Section 3.3.7
CRS	Sulphide-selective electrode	Section 3.3.7
Porewater THg	Tin [II] chloride reduction, CV-AFS	Section 3.4.1
Porewater MeHg	Distillation, CVAFS	Section 3.4.2
Cl^-	Ion chromatography	Section 3.4.3
SO_4^{2-}	Ion chromatography	Section 3.4.3
Fe[II]	UV spectrometer	Section 3.4.4
Fe	ICP-OES	Section 3.4.5
Mn	ICP-OES	Section 3.4.5
S^{2-}	Sulphide-selective electrode	Section 3.4.6

Soil-water partition coefficients (K_D) were calculated as:

$$K_D = \frac{P}{C}$$

where P is the solid-phase concentration and C is the porewater concentration. Partition coefficients were calculated for THg, MeHg, Fe and Mn.

The MeHg to THg concentration ratio (% MeHg) in sediment and porewater was calculated in this study and is widely used in the literature as a proxy for the relative Hg methylation rate (Bloom *et al*. 1999, Choe *et al*. 2004, Fitzgerald *et al*. 2007), however % MeHg should be used with caution as it is not a direct measure of Hg methylation rates.

Sulphate/chloride quotients were also calculated to give an indication of salinity. Seawater typically has a SO_4^{2-}/Cl^- quotient of 0.05 (Kolditz *et al*. 2009) and can be useful to determine whether sulphate is being utilised by SRB, indicated by a lower ratio, or whether reduced sulphide species are being oxidised, indicated by a higher SO_4^{2-}/Cl^- ratio (Kolditz *et al*. 2009).

Aluminium-normalised enrichment factors are calculated to give an indication of the overall contamination loading of Hg compared to baseline concentrations. The enrichment factor (EF) is calculated according to the following equation:

$$EF = \frac{(Hg_x/Al_x)}{(Hg_b/Al_b)}$$

where Hg_x and Al_x are the sediment sample concentrations Hg and Al, while Hg_b and Al_b are their background concentrations. An EF value between 0.5 and 1.5 suggests that the Hg may entirely originate from crustal materials. An EF value greater than 1.5 indicates that a significant portion of the Hg is derived from non-crustal materials, i.e. from a point or non-point pollution source (Gargouri *et al.* 2011).

6.2 Statistical Analysis

Statistical analyses were performed using SPSS 19.0 and R 3.1.1 (R Development Core Team, 2013) for Windows. Before any statistical analysis was completed the distribution of the data was determined. Dytham (2011) states that significance tests for a normal distribution, such as the Kolmogorov-Smirnov test, are more precise although should be used with caution if there are fewer than 50 observations. This study contains 60 observations and so the Kolmogorov-Smirnov test was applied to this study (Table 6.2).

Transformed data (Table 6.2) were analysed using a two-way analysis of variance (ANOVA) to determine differences between sites (Orplands, Northey Island and Ferry Lane) and site-type (restored or natural) as well as identifying any interaction between site and site-type. A Bonferroni post-hoc test was carried out on sites where the two-

way ANOVA gave a significant result. Groups of data were considered significantly different at $p < 0.05$.

Spearman's rank correlation analyses were used to test the association between THg and MeHg concentrations and physico-chemical variables. Relationships between Hg and physico-chemical variables may indicate the influence of these variables on Hg methylation and distribution. No outlying data were removed as this was an exploratory analysis. Relationships were considered statistically significant at $p < 0.05$. Spearman's correlation rather than Pearson's correlation was used because MeHg partitioning data could not be successfully transformed to a normal distribution.

The multivariate test of Principal Components Analysis (PCA) was used as an exploratory method to help identify any underlying controls on the data.

Table 6.2

Kolmogorov-Smirnov test for normality

		Significance	Transformation	Significance after transformation
Sediment	THg	0.474	-	-
	MeHg	0.046	log(Y)	0.868
	% MeHg	0.000	log(Y)	0.606
	LOI	0.675	-	-
	pH	0.954	-	-
	AVS	0.030	log(Y)	0.607
	CRS	0.005	log(Y)	0.638
	Fe	0.186	-	-
	Mn	0.001	log(Y)	0.614
Porewater	THg	0.015	log(Y)	0.729
	MeHg	0.006	log(Y)	0.178
	% MeHg	0.068	-	-
	Fe	0.000	log(Y)	0.178
	Fe[II]	0.000	log(Y)	0.205
	Mn	0.000	1/sqrt(Y)	0.061
	H_2S	0.000	1/sqrt(Y)	0.193
	SO_4^{2-}	0.835	-	-
	Cl^-	0.477	-	-
Partition Coefficients	log K_D THg	0.764	-	-
	log K_D MeHg	0.021	No successful transformation	
	log K_D Fe	0.168	-	-

6.2.1 Principal Components Analysis (PCA)

Principal components analysis is an exploratory method used to identify clusters of correlations in multidimensional space, which may explain underlying controls on the data (Field 2009). It is particularly useful for large datasets because PCA reduces a dataset to a more manageable size whilst retaining as much of the original information as possible (Webster 2001, Field 2009). The main goal of PCA is to provide an easy visualisation of the relationships that exist amongst the variables determined in large data sets (Passos *et al*. 2010).

A correlation matrix was used for data analysis because variables in the dataset are measured using different units and a correlation matrix standardises the variables so the variances are the same (Webster 2001). The correlation coefficients between the original variables and the PCs are called factor loadings and are used to determine the influence of each variable on that factor (Reid and Spencer 2009). Factor loadings are considered to affect the factor significantly if the value is above 0.6 (Passos *et al*. 2010), although Field (2009) argues that values above 0.4 should be considered. Component scores are used to identify correlations between variables and MeHg data. To aid and improve data interpretation, PCs can be rotated such that variables are loaded maximally on only one factor (Scrimshaw and Lester 2001). An orthogonal rotation (Varimax method) was used here because it assumes that the PCs are uncorrelated and the components are rotated while keeping the axes perpendicular. This ensures the PCs remain independent (Field 2009).

Various data pre-treatments can be applied to improve the interpretation of PCA (Reid and Spencer 2009). Here, three different datasets were analysed to investigate the effect of pre-treatments on the dataset. These were raw datasets, geochemically normalised datasets, and transformed datasets. Transformations are applied to obtain a normal distribution, standardise the dataset, and reduce the influence of outliers (Reid and Spencer 2009). The geochemically normalised dataset consisted of metal data normalised to Al concentrations. Metals occur naturally in sediments and their concentrations vary with sediment type and grain size (Schropp *et al*. 1990).

Normalisation is the attempt to compensate for this natural variability so that other factors controlling metal concentrations can be determined. It also enables the physico-chemical controls to be examined because they will not be masked by grain size. There are various data treatments used to compensate for grain size variability including granulometric and geochemical methods. Geochemical normalisation (i.e., against Al, as used here) is preferred over granulometric methods because it compensates for the mineralogical and the natural granular variability of trace-metal concentrations in sediments (Loring 1991).

A PCA of the raw and transformed data sets were both dominated by grain size variability and did not provide any further explanation into the controls on Hg variability and methylation. Therefore the best interpretation using PCA was offered by correcting for the influence of grain size and the geochemically normalised dataset is presented here.

6.3 Results

6.3.1 Total Hg, MeHg and % MeHg in sediments

Average THg, MeHg and % MeHg in surface sediment at the six study sites are shown in Figure 6.1 and Table 6.3. All THg and MeHg sediment concentrations are expressed per dry mass of sediment. Total Hg concentrations ranged from 10 to 201 ng g^{-1}. A two-way ANOVA was conducted that examined the effect of site and site-type on THg concentrations. There was a statistically significant interaction between the effects of site and site-type on THg (Table 6.4). THg concentrations were higher in the natural sites at Ferry Lane and Northey Island, whereas at Orplands THg concentrations were higher at the restored site.

Methylmercury concentrations ranged from 167 to 5456 pg g^{-1} and showed less variability than THg data across sites. There were no significant differences between site, site-type or interactions between site and site-type (Table 6.4). The MeHg to THg concentration ratio (% MeHg) showed similar patterns to MeHg concentrations (Table 6.3).

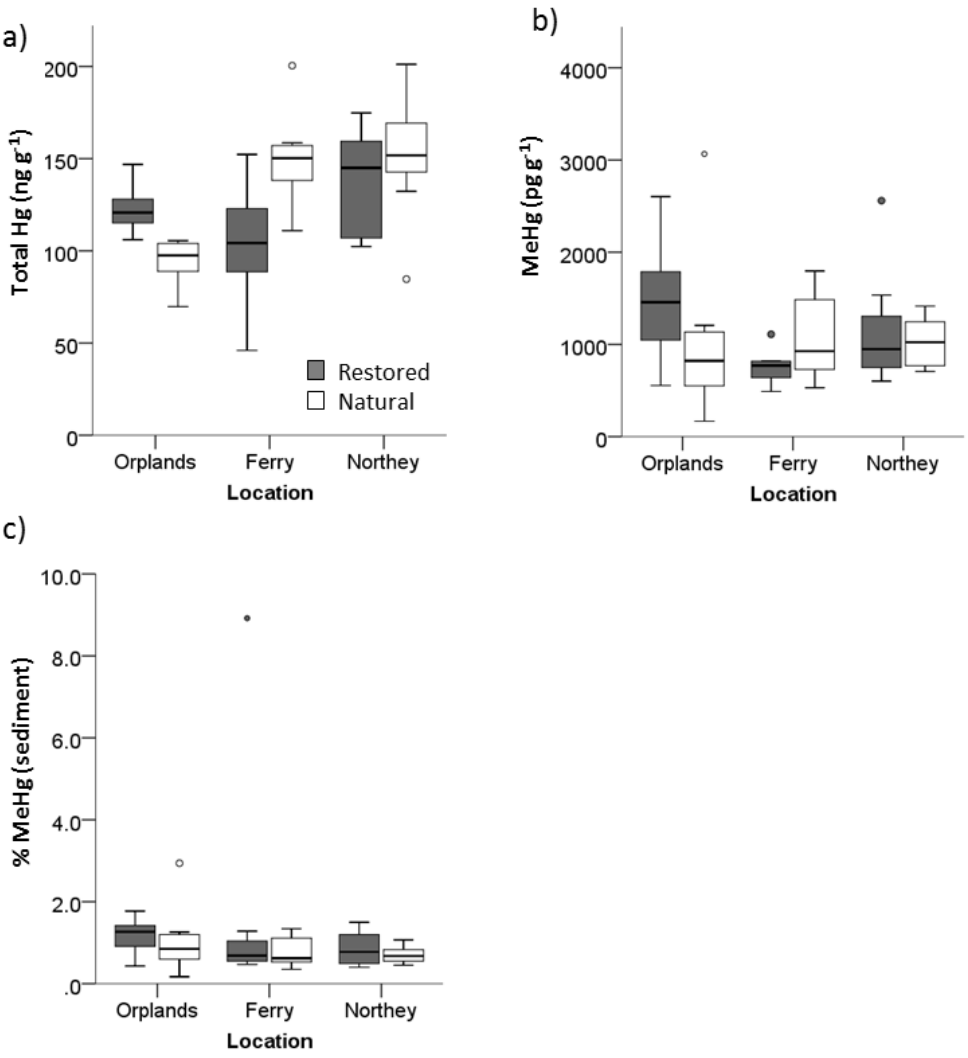

Figure 6.1 Sediment (a) THg (ng g^{-1}), (b) MeHg (pg g^{-1}), and (c) % MeHg for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 6.3

Average sediment total Hg (THg), methylmercury (MeHg), and % MeHg concentrations. Range is given in parentheses.

	THg ($ng\ g^{-1}$)		MeHg ($pg\ g^{-1}$)		% MeHg	
Orplands						
Restored	122	(10.2-147)	1450	(555-2600)	1.18	(0.43-1.77)
Natural	92.3	(69.8-106)	986	(167-3070)	1.02	(0.17-2.94)
Ferry Lane						
Restored	104	(46.0-152)	1220	(490-5460)	1.56	(0.47-8.92)
Natural	149	(111-201)	1080	(530-1800)	0.74	(0.35-1.34)
Northey						
Restored	138	(102-175)	1140	(602-2560)	0.85	(0.40-1.50)
Natural	151	(84.7-201)	1030	(706-1420)	0.70	(0.45-1.07)

Table 6.4
Two-Way ANOVA results for sediment geochemical data

Factors	*df*	MS	F-statisitc	*p*-value
THg				
Site	2	6436	10.8	< 0.001
Type	1	1671	2.80	0.100
Interaction	2	6470	10.8	< 0.001
MeHg				
Site	2	0.00	0.08	0.927
Type	1	0.08	1.41	0.240
Interaction	2	0.11	1.93	0.156
% MeHg				
Site	2	0.07	1.07	0.350
Type	1	0.18	2.60	0.113
Interaction	2	0.01	0.17	0.844
LOI				
Site	2	80.8	7.05	0.002
Type	1	22.0	1.92	0.171
Interaction	2	78.9	6.88	0.002
pH				
Site	2	0.24	8.03	0.001
Type	1	0.01	0.38	0.541
Interaction	2	0.16	5.38	0.007
AVS				
Site	2	0.73	19.7	< 0.001
Type	1	0.08	2.29	0.136
Interaction	2	0.07	1.89	0.161
CRS				
Site	2	1.07	24.7	< 0.001
Type	1	0.26	5.92	0.018
Interaction	2	0.11	2.52	0.090
Fe				
Site	2	215145970	6.49	0.003
Type	1	367090898	11.1	0.002
Interaction	2	136200997	4.11	0.022
Mn				
Site	2	0.38	12.9	< 0.001
Type	1	0.37	12.3	0.001
Interaction	2	0.07	2.42	0.099

6.3.2 Sediment geo-chemical parameters: LOI, pH, AVS, CRS, Fe and Mn

Average LOI, pH, AVS, CRS, Fe and Mn concentrations in surface sediment at the six study sites are shown in Figure 6.2 and Table 6.5. LOI ranged from 10.3 to 30.2 %. There was a significant interaction between the effects of site and site-type on LOI (Table 6.4). LOI was higher in the natural sites at Orplands and Ferry Lane, whereas LOI was higher in the restored site at Northey Island.

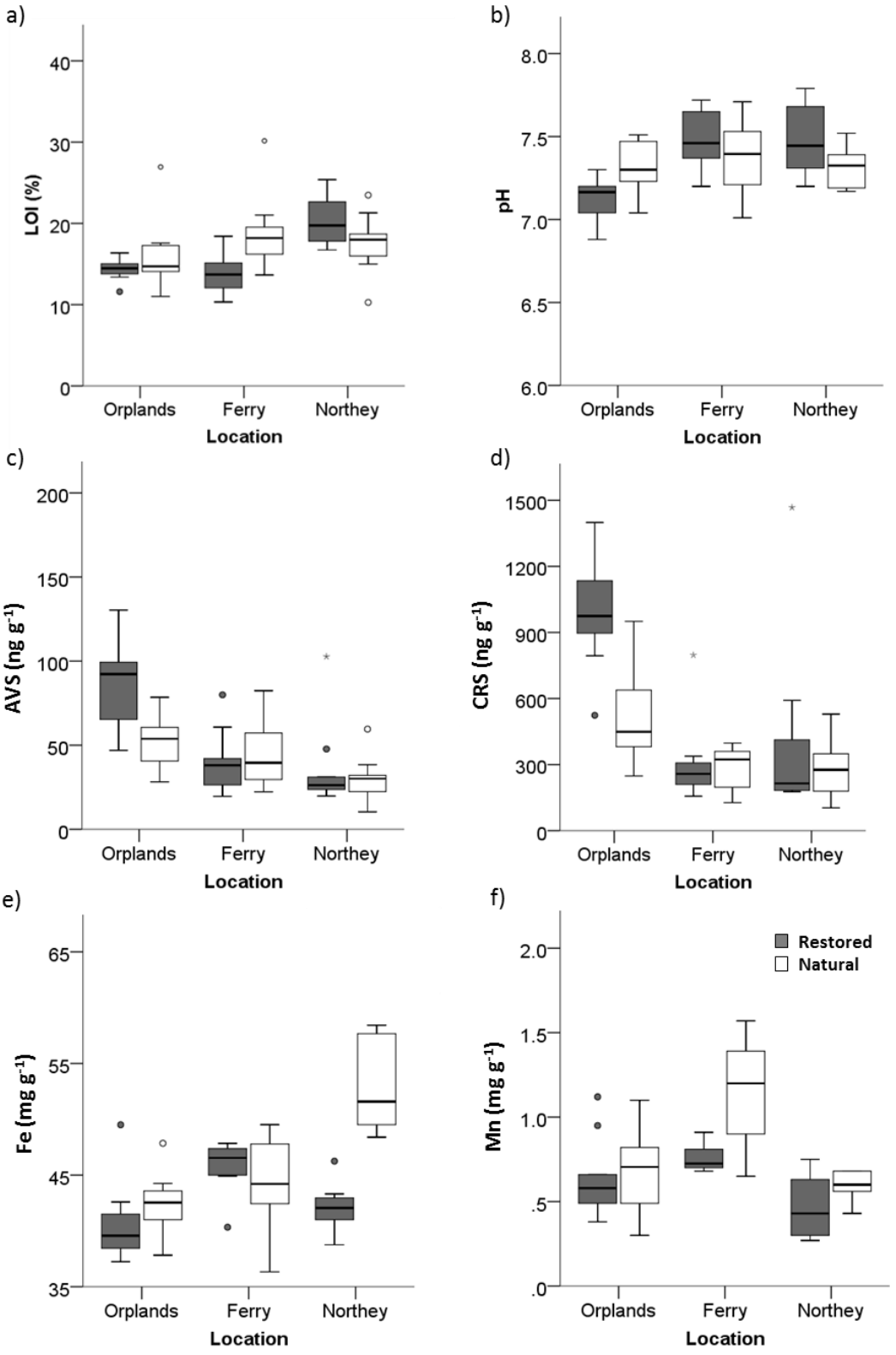

Figure 6.2 Sediment (a) LOI (%), (b) pH, (c) AVS (ng g^{-1}), (d) CRS (ng g^{-1}), (e) Fe (mg g^{-1}), and (f) Mn (mg g^{-1}) for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 6.5
Average sediment geochemical parameters. Range is given in parentheses.

	LOI (%)		pH		AVS (ng g^{-1})		CRS (ng g^{-1})		Fe (mg g^{-1})		Mn (mg g^{-1})	
Orplands												
Restored	14.4	(11.6-16.4)	7.13	(6.88-7.30)	87.3	(47.0-130)	991	(524-1400)	40.6	(37.2-49.5)	0.64	(0.38-1.12)
Natural	15.8	(11.0-27.0)	7.31	(7.04-7.51)	59.0	(28.2-135)	521	(248-951)	42.5	(37.8-47.9)	0.67	(0.30-1.10)
Ferry Lane												
Restored	13.8	(10.3-18.4)	7.47	(7.20-7.72)	40.1	(19.6-80.0)	307	(157-797)	45.9	(40.3-47.8)	0.77	(0.68-0.91)
Natural	18.9	(13.7-30.2)	7.36	(7.01-7.71)	45.0	(22.3-82.4)	281	(127-398)	47.9	(36.3-81.8)	1.62	(0.65-6.03)
Northey												
Restored	20.4	(16.7-25.4)	7.48	(7.20-7.79)	35.5	(19.8-103)	390	(177-1467)	42.0	(38.8-46.2)	0.45	(0.27-0.75)
Natural	17.6	(10.3-23.5)	7.32	(7.17-7.52)	28.9	(10.4-59.6)	284	(103-529)	53.0	(48.4-58.4)	0.76	(0.43-1.61)

pH ranged from 6.88 to 7.79 (Table 6.5). There was a significant interaction between the effects of site and site-type on pH (Table 6.4). pH was higher in the restored sites at Ferry Lane and Northey Island, whereas pH was lower in the restored site at Orplands.

AVS and CRS concentrations showed high variation across sites with highest concentrations at Orplands restored site (mean of 87 and 991 ng g^{-1} respectively). Northey Island natural site contained lowest concentrations (mean of 35 and 390 ng g^{-1} respectively). The two-way ANOVA for AVS showed significant differences between sites. There were no significant differences between site-type or significant interactions between site and site-type. The Bonferroni post-hoc test showed significant difference between Orplands and Ferry Lane, as well as between Orplands and Northey Island, but no significant difference between Ferry Lane and Northey (Table 6.6).

Table 6.6
Bonferroni post-hoc test for Two-Way ANOVA results from sediment samples

Factors		*p*-value
AVS	Orplands v Ferry	0.001
	Orplands v Northey	0.000
	Ferry v Northey	0.075
CRS	Orplands v Ferry	0.000
	Orplands v Northey	0.000
	Ferry v Northey	1.000
Mn	Orplands v Ferry	0.001
	Orplands v Northey	0.978
	Ferry v Northey	0.000

The two-way ANOVA for CRS also showed significant differences between sites and site-type (restored or natural). There was no significant interaction between site and site-type. The Bonferroni post-hoc test showed significant difference between

Orplands and Ferry Lane, as well as between Orplands and Northey Island but no significant difference between Ferry Lane and Northey (Table 6.6).

Total sediment Fe ranged from 36.3 to 81.8 mg g^{-1} (Table 6.5). A two-way ANOVA showed that there was a significant interaction between the effects of site and site-type on Fe concentrations (Table 6.4). Fe was higher in the natural sites at all three sites, however the difference in Fe concentrations at the restored and natural site was more pronounced at Northey Island.

Total Mn concentrations showed high variability across sites. There were significant differences between site and site-type but no significant interaction between site and site-type (Table 6.4). Mn concentrations were higher in natural site at all three locations. Bonferroni post-hoc test showed significant differences between Orplands and Ferry Lane, as well as between Ferry Lane and Northey Island. There was no significant difference between Orplands and Northey Island (Table 6.6).

6.3.3 Total Hg, MeHg and Hg methylation (% MeHg) in porewater

Total Hg, MeHg and % MeHg in surface sediment porewater at the six study sites are shown in Figure 6.3 Table 6.7. Total Hg concentrations in porewater ranged from 3.9 to 66 ng L^{-1} and showed high variability across sites, with highest concentrations in Orplands and Ferry Lane restored sites (mean of 18.2 and 18.82 ng L^{-1} respectively). THg concentrations were significantly higher in restored sites (Table 6.8). There were no significant differences in THg concentrations between sites or interactions between site and site-type.

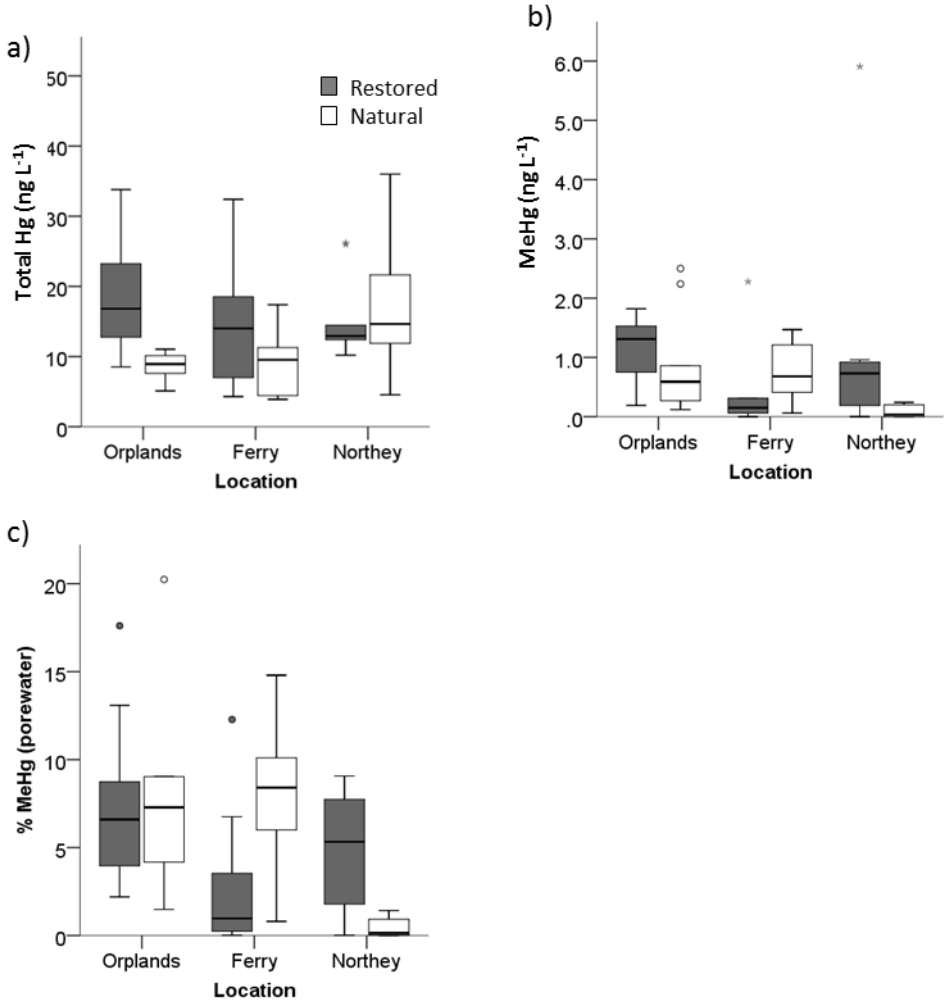

Figure 6.3 Porewater (a) THg (ng L^{-1}), (b) MeHg (ng L^{-1}), and (c) % MeHg for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 6.7
Average porewater total Hg (THg) and methylmercury (MeHg) concentrations and geochemical parameters. Range is given in parentheses.

	THg ($ng\ L^{-1}$)		MeHg ($ng\ L^{-1}$)		% MeHg	
Orplands						
Restored	18.2	(8.53-33.8)	1.18	(0.19-1.82)	7.33	(2.19-17.6)
Natural	8.65	(5.09-11.1)	0.84	(0.12-2.50)	8.95	(1.48-25.3)
Ferry Lane						
Restored	18.8	(4.29-65.9)	0.37	(0.00-2.28)	2.71	(0.02-12.3)
Natural	9.19	(3.90-17.4)	0.76	(0.06-1.47)	8.15	(0.80-14.8)
Northey						
Restored	15.2	(10.2-26.2)	1.08	(0.00-5.91)	6.23	(0.02-22.8)
Natural	17.2	(4.55-36.0)	0.08	(0.00-0.24)	0.43	(0.01-1.42)

Table 6.8
Two-Way ANOVA results for porewater Hg data

Factors	*df*	MS	F-statisitc	*p*-value
THg				
Site	2	0.11	2.14	0.128
Type	1	0.42	8.29	0.006
Interaction	2	0.14	2.75	0.073
MeHg				
Site	2	4.32	8.66	0.001
Type	1	1.11	2.23	0.141
Interaction	2	5.15	10.3	0.000
% MeHg				
Site	2	116	4.33	0.018
Type	1	2.66	0.10	0.754
Interaction	2	163	6.07	0.004

MeHg concentrations ranged from 0 to 5.91 ng L^{-1} and % MeHg ranged from 0.01 to 25.25 % (mean of 5.63 %; Table 6.7). A two-way ANOVA showed that there was a statistically significant interaction between the effects of site and site-type on MeHg concentrations and % MeHg (Table 6.8). MeHg concentrations and % MeHg are most different between restored and natural sites at Northey Island.

6.3.4 Porewater geo-chemical parameters: Mn, Fe, Fe[II], S^{2-}, SO_4^{2-}/Cl^-

Average Mn, Fe, Fe[II], S^{2-} and SO_4^{2-}/Cl^- in surface porewater at the six study sites are shown in Figure 6.4 and Table 6.9. Mn ranged from 0.05 to 20.47 mg L^{-1}, Fe concentrations ranged from 0.16 to 17.60 mg L^{-1} and Fe[II] concentrations ranged from 0.03 to 10.25 mg L^{-1}. A two-way ANOVA showed that there was a statistically significant interaction between the effects of site and site-type on Mn, Fe and Fe[II] concentrations (Table 6.10). Mn, Fe and Fe[II] was lower in the natural sites at Orplands and Northey, but higher in the natural site at Ferry Lane.

Sulphide concentrations ranged from 0.04 to 0.94 mg L^{-1}. There was a statistically significant interaction between the effects of site and site-type on S^{2-} concentrations (Table 6.10). S^{2-} was higher in the natural sites at all three sites, however the difference in S^{2-} concentrations at the restored and natural site was more pronounced at Orplands.

Sulphate/Chloride ratios ranged from 0.130 to 0.165. There were significant differences between both site and site-type, but no significant interaction between them (Table 6.10). Bonferroni post hoc test showed significant differences between Orplands and Northey Island (p = 0.029). There was no significant difference between Orplands and Ferry Lane or Ferry Lane and Northey Island.

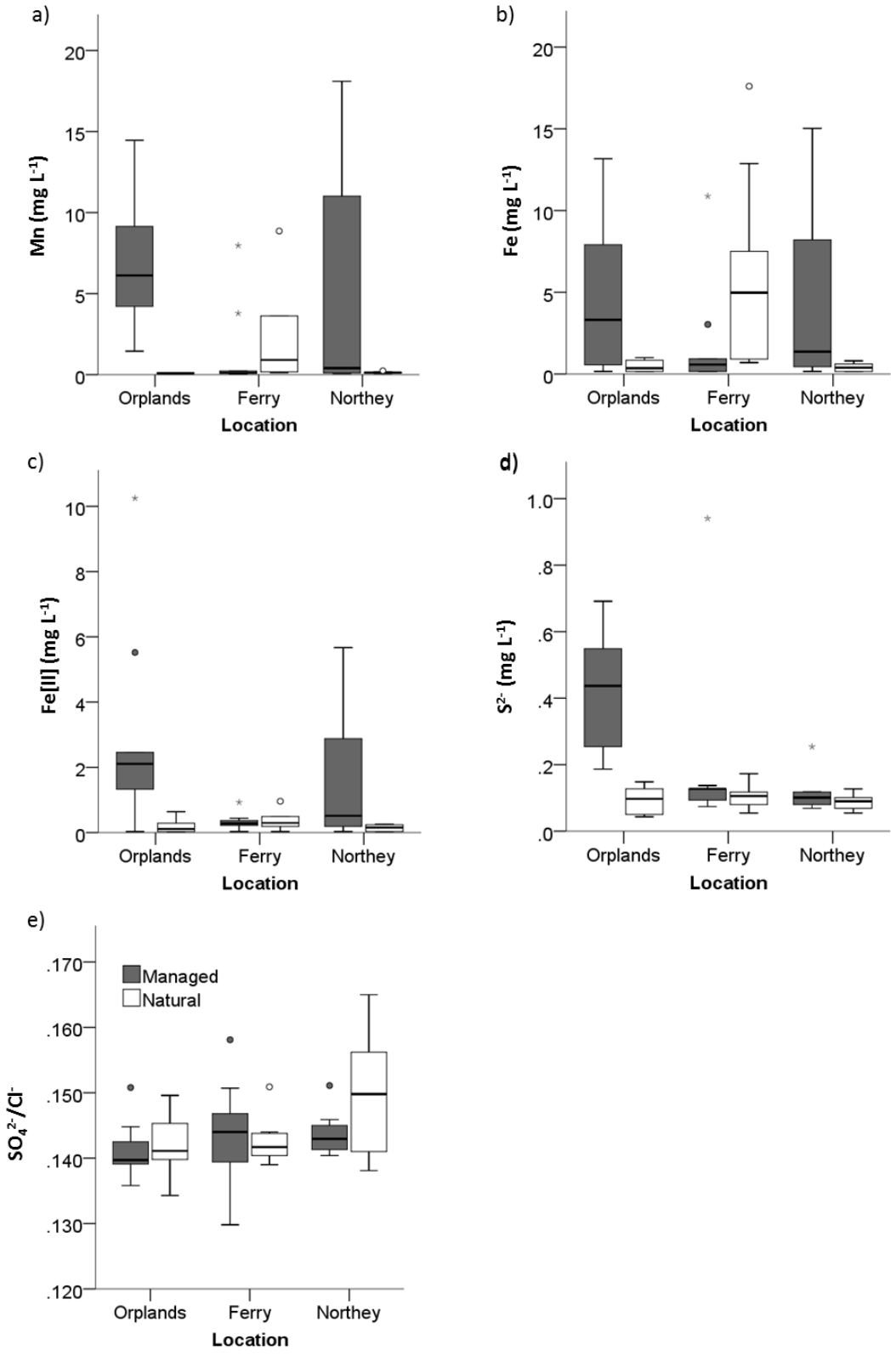

Figure 6.4 Porewater (a) Mn (mg L^{-1}), (b) Fe (mg L^{-1-}), (c) Fe[II] (mg L^{-1}), (d) S^{2-} (mg L^{-1}), and (e) SO_4^{2-}/Cl^- for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 6.9

Average porewater total Hg (THg) and methylmercury (MeHg) concentrations and geochemical parameters. Range is given in parentheses.

	Mn		Fe		Fe[II]		S^{2-}		SO_4^{2-}/Cl^-	
	(mg L^{-1})		(mg L^{-1})		(mg L^{-1})		(mg L^{-1})		(mg L^{-1})	
Orplands										
Restored	7.09	(1.45-14.5)	4.70	(0.16-13.2)	2.90	(0.03-10.3)	0.416	(0.19-0.69)	0.141	(0.136-0.151)
Natural	0.17	(0.05-0.93)	0.99	(0.16-6.22)	0.18	(0.03-0.64)	0.091	(0.04-0.15)	0.142	(0.134-0.150)
Ferry Lane										
Restored	1.26	(0.05-7.97)	1.75	(0.16-10.9)	0.34	(0.03-0.93)	0.193	(0.07-0.94)	0.144	(0.130-0.158)
Natural	3.75	(0.12-20.5)	6.04	(0.70-17.6)	0.73	(0.03-4.22)	0.103	(0.05-0.17)	0.143	(0.139-0.151)
Northey										
Restored	4.57	(0.07-18.1)	4.10	(0.16-15.0)	1.72	(0.03-5.67)	0.111	(0.07-0.25)	0.144	(0.140-0.144)
Natural	0.13	(0.07-0.24)	0.43	(0.16-0.81)	0.14	(0.03-0.26)	0.088	(0.05-0.13)	0.150	(0.138-0.165)

Table 6.10
Two-Way ANOVA results for porewater geochemical data

Factors	*df*	MS	F-statisitc	*p*-value
Mn				
Site	2	1.14	1.07	0.351
Type	1	11.1	10.4	0.002
Interaction	2	24.5	23.1	0.000
Fe				
Site	2	0.50	1.55	0.223
Type	1	0.55	1.68	0.200
Interaction	2	3.36	10.4	0.000
Fe[II]				
Site	2	0.26	0.74	0.483
Type	1	5.55	16.0	0.000
Interaction	2	2.23	6.43	0.003
S^{2-}				
Site	2	0.09	6.62	0.003
Type	1	0.39	30.2	0.000
Interaction	2	0.14	11.2	0.000
SO_4^{2-}/Cl^-				
Site	2	0.01	4.16	0.021
Type	1	0.01	4.07	0.049
Interaction	2	0.00	0.70	0.501

6.3.5 Partition Coefficients

Total Hg partition coefficients (log K_D) ranged from 3.24 to 4.61 L kg^{-1} (Figure 6.5a) and are consistently higher in the natural sites compared to the restored sites. There are significant differences between site-type and site (Table 6.11), however no significant interaction between them. A Bonferroni post hoc test showed significant differences between Ferry Lane and Northey Island (Table 6.12). There was no significant difference between Orplands and Ferry Lane or Orplands and Northey Island.

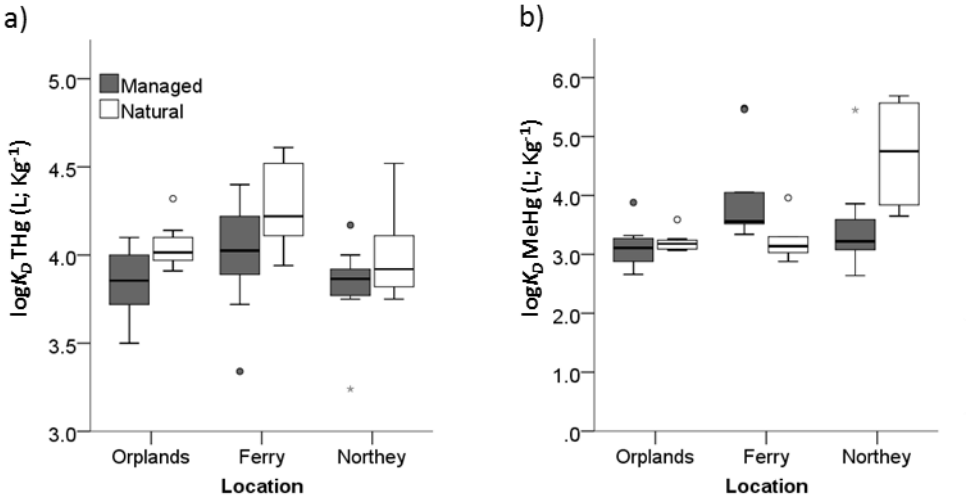

Figure 6.5 (a) logK_D THg (L kg^{-1}) and (b) logK_D MeHg (L kg^{-1}) for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

Table 6.11
Two-Way ANOVA results for THg partitioning data

Factors	*df*	MS	F-statisitc	*p*-value
THg				
Site	2	0.26	5.11	0.009
Type	1	0.61	11.9	0.001
Interaction	2	0.01	0.28	0.756

Table 6.12
Bonferroni post-hoc test for Two-Way ANOVA results from THg partitioning data

Factors		*p*-value
THg	Orplands v Ferry	0.057
	Orplands v Northey	1.000
	Ferry v Northey	0.011

MeHg partition coefficients (log K_D) ranged from 2.62 to 5.69 L kg^{-1} (Figure 6.5b) and a Kruskal-Wallis test was performed as the data are non-parametric. Methylmercury K_D are highest at Northey Island natural site (mean of 4.71 L kg^{-1}) and are significantly higher than Northey restored site (mean of 3.44 L kg^{-1}; Kruskal–Wallis test, ChiSquare (χ^2) = 9.849, df = 1, $p < 0.01$). Ferry Lane restored site had significantly higher MeHg K_D than the natural site (Kruskal–Wallis test, ChiSquare (χ^2) = 7.005, df = 1, $p < 0.01$). Orplands restored and natural site had the lowest MeHg K_D values and there were no significant differences between the two sites.

6.3.6 The relationship between THg and MeHg, and physico-chemical parameters.

Outputs from the Spearman's rank correlation analyses are shown in Figure 6.6 whilst key bivariate plots which demonstrate the associations are shown in Figure 6.7 and Figure 6.8. There was a positive association between THg and LOI (r = 0.340, $p < 0.01$). Sediment MeHg also has a positive relationship with porewater THg (r = 0.318, p <0.05). Significant correlations were also evident between sediment MeHg and pH (r = -0.330, $p < 0.01$), AVS (r = 0.360, p <0.01), and Fe[II] (r = 0.330, $p < 0.01$). The sediment MeHg to THg concentration ratio (% MeHg) was strongly correlated with AVS (r = 0.474, $p < 0.001$) and porewater sulphide (r = 0.466, $p < 0.001$).

Porewater MeHg concentration correlated strongly with similar physio-chemical parameters to sediment MeHg concentrations. There were strong positive correlations between porewater MeHg and indicators of reducing conditions i.e., Fe[II] (r = 0.561, p <0.001) and porewater sulphide (r = 0.493, $p < 0.001$). Porewater MeHg concentrations also correlated negatively with SO_4^{2-}/Cl^- (r = -0.346, $p < 0.01$), and the partition coefficient for Fe (r = -0.671, $p < 0.001$).

			SEDIMENT									POREWATER								PARTITIONING COEFFICIENTS		
			THg ($ng\ g^{-1}$)	MeHg ($pg\ g^{-1}$)	%MeHg	%LOI	pH	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	AVS ($\mu mol\ g^{-1}$)	CRS ($\mu mol\ g^{-1}$)	THg ($ng\ L^{-1}$)	MeHg ($ng\ L^{-1}$)	%MeHg	Fe ($mg\ L^{-1}$)	Mn ($mg\ L^{-1}$)	Fe[II] ($mg\ L^{-1}$)	H_2S (μM)	SO_4^{2-}/Cl^-	$logK_D$ THg ($L\ Kg^{-1}$)	$logK_D$ MeHg ($L\ Kg^{-1}$)	$logK_D$ Fe ($L\ Kg^{-1}$)
SEDIMENT	THg ($ng\ g^{-1}$)	Spearman's	1.0	0.142	**-0.381**	**0.343**	-0.003	**0.277**	-0.209	-0.203	-0.163	0.158	-0.164	-0.194	**0.257**	0.237	0.083	-0.006	0.081	0.036	0.186	-0.204
		Sig.		0.278	**0.003**	**0.007**	0.983	**0.032**	0.109	0.120	0.213	0.227	0.210	0.137	**0.048**	0.069	0.530	0.966	0.536	0.788	0.155	0.117
	MeHg ($pg\ g^{-1}$)	Spearman's		1.0	**0.807**	0.081	**-0.330**	-0.072	0.002	**0.360**	0.154	**0.318**	**0.511**	**0.383**	**0.408**	**0.473**	**0.330**	**0.536**	-0.088	-0.222	-0.114	**-0.402**
		Sig.			**0.000**	0.537	**0.010**	0.586	0.986	**0.005**	0.240	**0.013**	**0.000**	**0.003**	**0.001**	**0.000**	**0.010**	**0.000**	0.504	0.088	0.387	**0.001**
	%MeHg	Spearman's			1.0	-0.093	**-0.301**	-0.187	0.112	**0.474**	**0.264**	**0.295**	**0.539**	**0.406**	0.229	**0.302**	**0.328**	**0.466**	-0.040	**-0.321**	-0.184	-0.244
		Sig.				0.482	**0.019**	0.152	0.392	**0.000**	**0.041**	**0.022**	**0.000**	**0.001**	0.078	**0.019**	**0.011**	**0.000**	0.764	**0.012**	0.158	0.060
	%LOI	Spearman's				1.0	0.043	-0.032	0.047	-0.087	-0.128	-0.006	0.067	0.136	0.132	-0.045	-0.070	**-0.258**	0.241	-0.021	-0.113	-0.123
		Sig.					0.746	0.810	0.719	0.509	0.329	0.963	0.609	0.300	0.314	0.735	0.596	**0.046**	0.064	0.873	0.389	0.348
	pH	Spearman's					1.0	0.220	-0.024	**-0.432**	**-0.435**	**-0.290**	**-0.407**	**-0.259**	-0.196	**-0.338**	**-0.284**	**-0.341**	0.044	0.234	**0.302**	0.242
		Sig.						0.092	0.855	**0.001**	**0.001**	**0.025**	**0.001**	**0.046**	0.134	**0.008**	**0.028**	**0.008**	0.737	0.072	**0.019**	0.062
	Fe ($mg\ kg^{-1}$)	Spearman's						1.0	0.239	**-0.361**	**-0.367**	0.005	**-0.418**	**-0.343**	-0.085	-0.226	-0.208	**-0.372**	**0.295**	0.223	**0.462**	0.206
		Sig.							0.066	**0.005**	**0.004**	0.972	**0.001**	**0.007**	0.517	0.083	0.111	**0.003**	**0.022**	0.087	**0.000**	0.115
	Mn ($mg\ kg^{-1}$)	Spearman's							1.0	-0.004	-0.155	-0.196	-0.024	0.107	0.007	-0.137	-0.196	-0.106	0.059	**0.273**	0.039	-0.005
		Sig.								0.973	0.236	0.133	0.856	0.417	0.955	0.297	0.134	0.419	0.652	**0.035**	0.766	0.967
	AVS ($\mu mol\ g^{-1}$)	Spearman's								1.0	**0.714**	0.193	**0.587**	**0.457**	**0.254**	**0.353**	**0.360**	**0.427**	-0.142	-0.137	**-0.448**	**-0.295**
		Sig.									**0.000**	0.140	**0.000**	**0.000**	**0.050**	**0.006**	**0.005**	**0.001**	0.279	0.297	**0.000**	**0.022**
	CRS (µmol g-1)	Spearman's									1.0	0.078	**0.413**	**0.301**	0.110	0.200	**0.340**	**0.338**	-0.017	-0.157	**-0.399**	-0.149
		Sig.										0.552	**0.001**	**0.019**	0.404	0.125	**0.008**	**0.008**	0.896	0.230	**0.002**	0.257
POREWATER	THg ($ng\ L^{-1}$)	Spearman's										1.0	0.170	-0.186	0.168	0.249	**0.422**	**0.329**	-0.027	**-0.854**	0.000	-0.142
		Sig.											0.194	0.156	0.201	0.055	**0.001**	**0.010**	0.836	**0.000**	0.999	0.279
	MeHg ($ng\ L^{-1}$)	Spearman's											1.0	**0.907**	**0.620**	**0.534**	**0.561**	**0.493**	**-0.346**	-0.151	**-0.869**	**-0.671**
		Sig.												**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.007**	0.250	**0.000**	**0.000**
	%MeHg	Spearman's												1.0	**0.565**	**0.410**	**0.393**	**0.305**	**-0.350**	0.177	**-0.846**	**-0.616**
		Sig.													**0.000**	**0.001**	**0.002**	**0.018**	**0.006**	0.177	**0.000**	**0.000**
	Fe ($mg\ L^{-1}$)	Spearman's													1.0	**0.680**	**0.724**	**0.388**	**-0.314**	0.022	**-0.501**	**-0.988**
		Sig.														**0.000**	**0.000**	**0.002**	**0.014**	0.869	**0.000**	**0.000**
	Mn ($mg\ L^{-1}$)	Spearman's														1.0	**0.651**	**0.579**	**-0.381**	-0.084	**-0.386**	**-0.687**
		Sig.															**0.000**	**0.000**	**0.003**	0.526	**0.002**	**0.000**
	Fe[II] ($mg\ L^{-1}$)	Spearman's															1.0	**0.504**	**-0.286**	**-0.292**	**-0.431**	**-0.727**
		Sig.																**0.000**	**0.026**	**0.024**	**0.001**	**0.000**
	H_2S (μM)	Spearman's																1.0	**-0.258**	**-0.263**	**-0.262**	**-0.422**
		Sig.																	**0.047**	**0.042**	**0.043**	**0.001**
	SO_4^{2-}/Cl^-	Spearman's																	1.0	0.013	**0.353**	**0.338**
		Sig.																		0.924	**0.006**	**0.008**
PARTITIONING COEFFICENTS	$logK_D$ THg ($L\ Kg^{-1}$)	Spearman's																		1.0	0.027	-0.016
		Sig.																			0.835	0.902
	$logK_D$ MeHg ($L\ Kg^{-1}$)	Spearman's																			1.0	**0.562**
		Sig.																				**0.000**
	$logK_D$ Fe ($L\ Kg^{-1}$)	Spearman's																				1.0
		Sig.																				

Figure 6.6. Spearman's rank correlation between THg and MeHg, and physico-chemical parameters

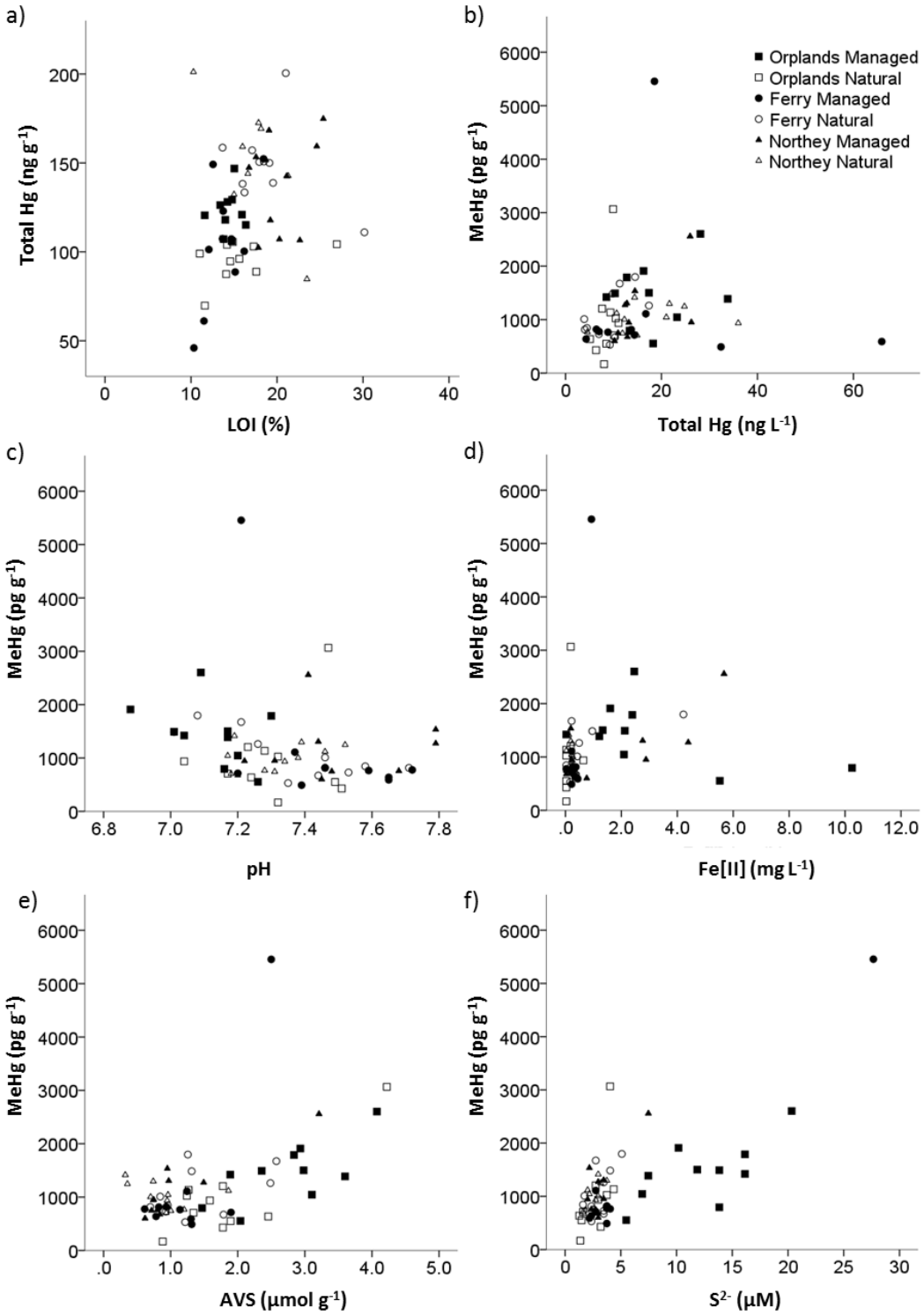

Figure 6.7 Bivariate plots of (a) sediment total Hg against loss on ignition, (b) sediment MeHg against porewater THg, (c) sediment MeHg against pH, (d) sediment MeHg against porewater Fe[II], (e) sediment MeHg against AVS, and (f) sediment MeHg against porewater sulphide.

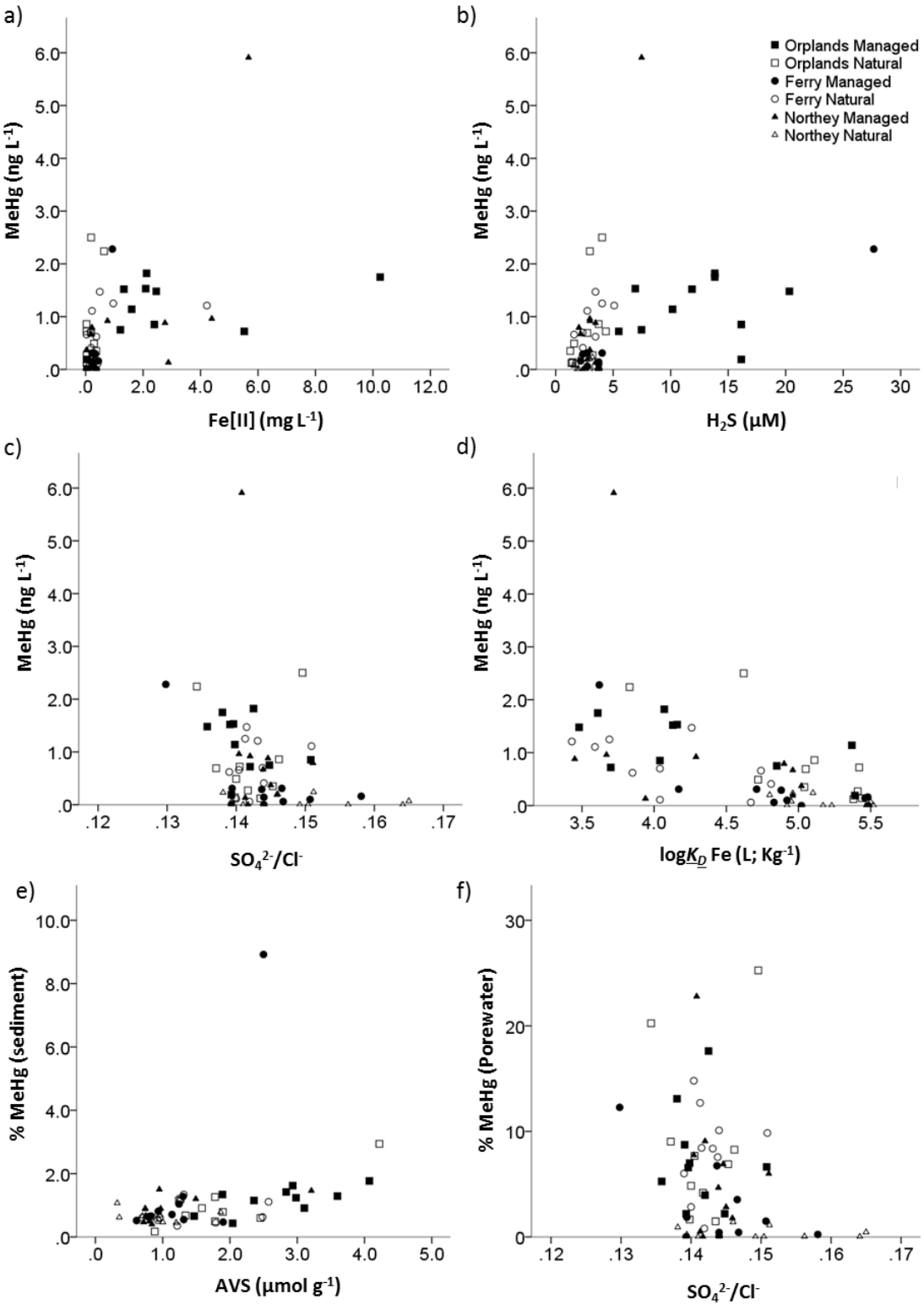

Figure 6.8 Bivariate plots of (a) porewater MeHg against porewater Fe[II}, (b) porewater MeHg against porewater sulphide, (c) porewater MeHg against SO_4^{2-}/Cl^-, (d) porewater MeHg against Fe partition coefficient, (e) sediment % MeHg against AVS, and (f) porewater % MeHg against SO_4^{2-}/Cl^-.

6.3.7 Principal Components Analysis

Principal component loadings for the geochemically normalised dataset are shown in Table 6.13. PCA was carried out as an exploratory exercise on potential predictor variables of MeHg concentrations. Principal Component analysis is a form of multidimensional scaling. It is a linear transformation of the variables into a lower dimensional space which retain maximal amount of information about the variables, producing scores for each principal component. Spearman's rank correlation analysis was then carried out to examine the association between principal component scores and THg and MeHg concentrations. By examining the variables that load onto the scores, and how strongly the scores correlate with THg and MeHg, the groups of variables that control MeHg variability is indicated. Correlations between PC scores, discussed below, are used to highlight how these controls on MeHg concentration change from between sites.

Four PCs were extracted, explaining a cumulative variance of 68 %. Principal component one (PC1) was highly loaded by metals including Mg, Cr, Cu, Pb, Ni, K, and to a lesser extent Fe. Principal component two (PC2) was strongly loaded by porewater Mn, Fe, Fe[II] and sulphide. pH was negatively loaded on PC2. The third principal component, PC3 is strongly loaded by Mn, Co, Cd, Fe, and to a lesser extent Ni. The fourth PC was highly loaded by sulphate, chloride, AVS and CRS.

6.3.7.1 Principal component scores and Hg sediment and porewater data

Spearman's rank correlations between scores for geochemically normalised data and Hg sediment and porewater data are shown in Table 6.14. The scores for PC1 correlate significantly with THg as well as porewater % MeHg and MeHg partition coefficients. The scores for PC2 correlate strongly with all Hg data, except THg partition coefficients. PC3 correlates significantly with both porewater and sediment MeHg concentrations, whilst PC4 correlates with MeHg porewater data.

Bivariate plots of the PC scores are shown in Figure 6.9. The sample site and site-type are indicated via different markers in an attempt to highlight how PC scores vary

depending on the site and site-type the sample was taken from. PC1, PC3 and PC4 show similar variability and there are no clear patterns in the spread of the data. PC2, however shows that Orplands managed site has higher values than all other sites.

Table 6.13
Factor loadings obtained from a PCA carried out on the geochemically normalised dataset.
Loadings above the critical threshold of 0.4 are shown, with those above 0.6 in bold.

	Factor 1	Factor 2	Factor 3	Factor 4
Variance explained	24%	16%	16%	12%
Mg/Al	**0.919**			
Cr/Al	**0.873**			
Cu/Al	**0.865**			
Pb/Al	**0.859**			
Ni/Al	**0.789**		0.46	
K/Al	**0.774**			
Mn (porewater)		**0.866**		
Fe (porewater)		**0.857**		
Fe[II] (porewater)		**0.768**		
Sulphide		**0.632**		
pH		-0.476		
Mn/Al			**0.944**	
Co/Al			**0.929**	
Cd/Al			**0.887**	
Fe/Al	0.592		**0.616**	
Sulphate				**0.806**
Chloride				**0.792**
CRS		0.501		**0.648**
AVS		0.533		0.573
Ca/Al				
LOI				

Table 6.14

Spearman's rank correlations between principal component scores and Hg sediment and porewater data.

		Component Score 1	Component Score 2	Component Score 3	Component Score 4
THg/Al	Spearman's *r*	**0.331**	**0.303**		
	p	**0.010**	**0.019**		
MeHg/Al	Spearman's *r*		**0.527**	**0.285**	
	p		**<0.001**	**0.027**	
% MeHg (sediment)	Spearman's *r*		**0.447**	**0.345**	
	p		**<0.001**	**0.007**	
THg ($ng\ L^{-1}$)	Spearman's *r*		**0.406**		
	p		**0.001**		
MeHg ($ng\ L^{-1}$)	Spearman's *r*		**0.658**	**0.255**	**0.282**
	p		**<0.001**	**0.049**	**0.029**
% MeHg (porewater)	Spearman's *r*	**0.329**	**0.484**	**0.292**	
	p	**0.010**	**<0.001**	**0.024**	
THg partition coefficient	Spearman's *r*				
	p				
MeHg partition coefficient	Spearman's *r*	**-0.326**	**-0.431**		
	p	**0.011**	**0.001**		

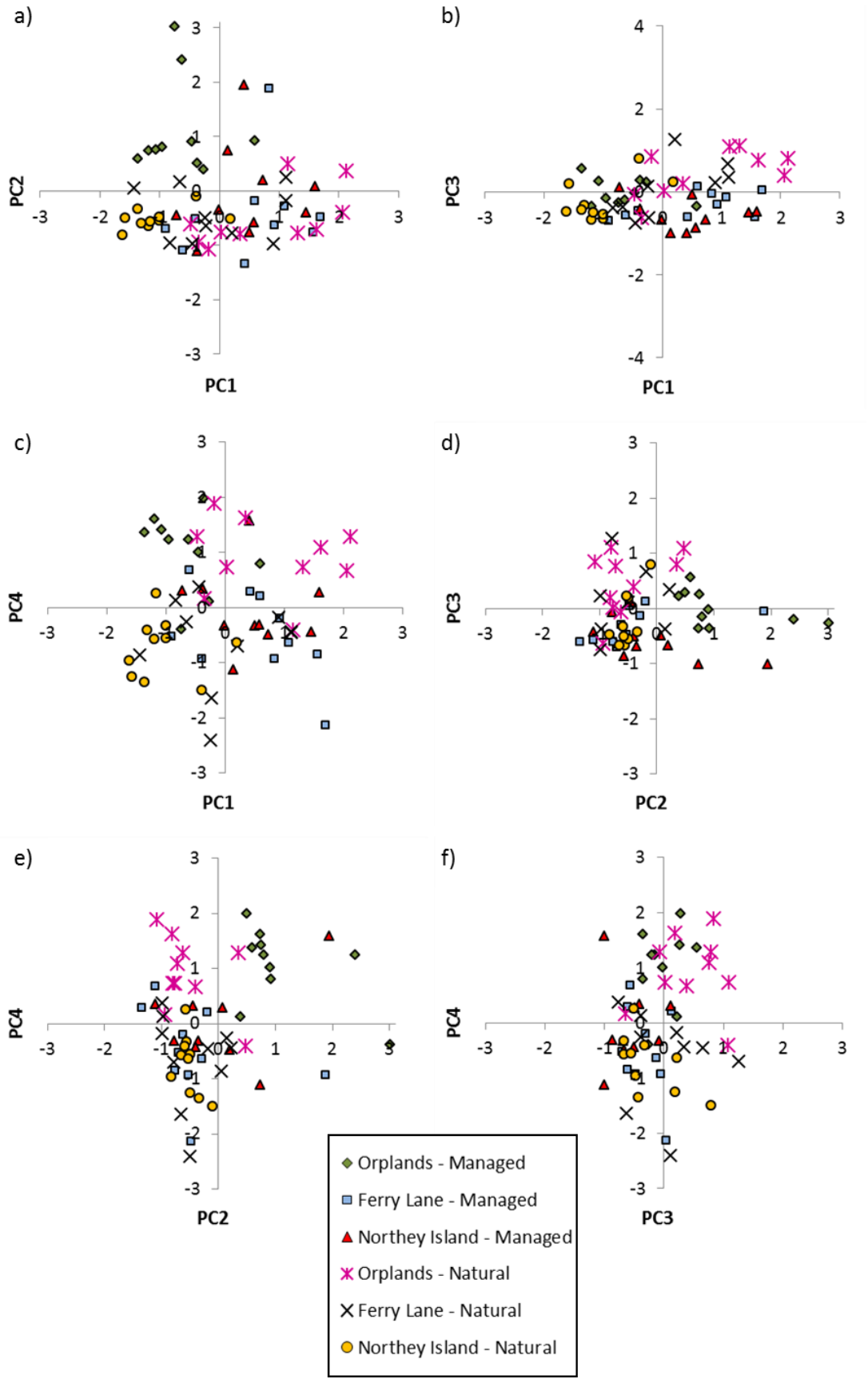

Figure 6.9 Bivariate plots between principal component scores

6.4 Discussion

6.4.1 Principal Component Analysis

The principal components and what they represent is first discussed before examining their correlation with THg and MeHg. The influence of mineralogy is suggested by the loadings on the first factor of the PCA explaining 24 % of the cumulative variance in the dataset. The scores for PC1 are loaded by Mg, Cr, Cu, Pb, Ni, K, and to a lesser extent Fe. Mg and K are indicative of clay mineralogy (Brindley and Brown 1984) and Fe has been shown to sorb and/or co-precipitate with clay minerals (Reid and Spencer 2009). Clay is a major component of the sediment in southeast England and therefore PC1 is attributed to the influence of the sediments natural mineralogy. Principal component two (PC2) was strongly loaded by porewater Mn, Fe, Fe[II] and sulphide which as discussed in Section 6.1 are environmental factors that indicate reducing conditions. AVS and CRS are also less strongly loaded onto PC2 which further indicates reducing conditions. Therefore, PC2 is attributed to redox conditions. Furthermore, pH is negatively loaded onto PC2. pH has been shown to decrease in reducing conditions as humic acid is released during anaerobic respiration (Blackwell *et al*. 2004).

The influence of anthropogenic sources was the next most important control on metal variability. PC3 is strongly loaded by Mn, Co, Cd, Fe, and to a lesser extent Ni. The lack of loadings by any other environmental or sediment composition such as sulphate, chloride or LOI suggests an anthropogenic origin (Reid and Spencer 2009). The fourth PC was highly loaded by sulphate, chloride, AVS and CRS. Sulphate and chloride are indicative of salinity as high sulphate and chloride concentrations are found in seawater. AVS and CRS concentrations can also increase in saline conditions due to the increase in reduced sulphate concentrations (Allen and Parkes 1995). Therefore, PC4 is attributed to salinity.

6.4.2 Total Hg and factors controlling its distribution

Overall, sediments in the sampling region contained relatively low levels of THg (10-201 ng g^{-1}) compared to other studies discussed in the literature (Conaway *et al*. 2003,

Hammerschmidt *et al*. 2004, Sunderland *et al*. 2004, Mitchell and Gilmour 2008, Morris *et al*. 2014; see Table 2.1). Hg concentration presented in Chapter 6 are significantly lower than those presented in Chapter 4. However, Chapter 4 measured composite Hg inputs over time whereas the sampling design (surface sediments) for Chapter 6 assessed recent inputs of Hg to the sites, reflecting more recent sediment and water quality conditions.

THg levels for most sampling sites are close to the natural background concentrations measured within the UK. Chapter 4 indicated that background levels are low (< 150 ng g^{-1}) although concentrations were still decreasing at depth and so background levels had not been reached. Background levels estimated from six lake sediment long cores collected across the UK (20 – 380 ng g-1, average of 120 ng g-1; Yang and Rose 2003) show that these surface sediments are only slightly contaminated.

Calculating normalised enrichment factors (EF) is a common approach to estimating the anthropogenic impact on sediments. The EF method normalises the Hg content with respect to a sample reference metal such as Al (Ravichandran et al. 1995). Metals occur naturally in sediments and their concentrations vary with grain size. Al is considered as a 'proxy' for grain size and normalising to Al compensates for this natural variability. Pre-industrial sediment concentrations from dated sediment cores collected in the Medway by Spencer (1999) were used as the Al background concentration. A background concentration of 40 mg g^{-1} for Al was used. Spencer (1999) did not provide a background value for Hg, and therefore the value for this metal was taken from O'Reilly Weise *et al*. (1995). O'Reilly Weise *et al*. (1995) presented background Hg concentrations from Essex saltmarshes and so the underlying geology will be similar to the sites used in this study. A background concentration of 100 ng g^{-1} for Hg was used (which is in agreement with the value estimated by Yang and Rose, 2003). Enrichment factors are given in Table 6.15.

Table 6.15 Enrichment factors from managed and natural sites

Site	Managed/Natural	Enrichment Factor
Orplands	Managed	1.66
	Natural	1.56
Ferry Lane	Managed	1.57
	Natural	1.74
Northey Island	Managed	1.97
	Natural	1.51

All sites contained enrichment factors greater than 1.5 indicating that at all sites a significant portion of Hg is derived from non-crustal materials. Instead, the Hg is provided by other sources, such as point and non-point pollution sources (Gargouri *et al.* 2011).

Total Hg concentrations showed high variability across sites with significant differences between sites (Figure 6.1). Spatial trends were less apparent after normalising to grain-size and LOI (Figure 6.10), indicating that grain size and LOI had a major control on THg distribution. Boxplots (normalised to Al and LOI) show that samples from all locations had similar variability between site type (restored or natural) and therefore controls on THg distribution are likely similar between natural and restored sites, and these controls do not appear to vary over time. THg seems to be largely controlled by sedimentology/mineralogy, and THg concentrations are indicative of natural background levels. The sediment sampled from all sites had likely been deposited in the previous 10 years and therefore finding no variability between sites or with time is not surprising.

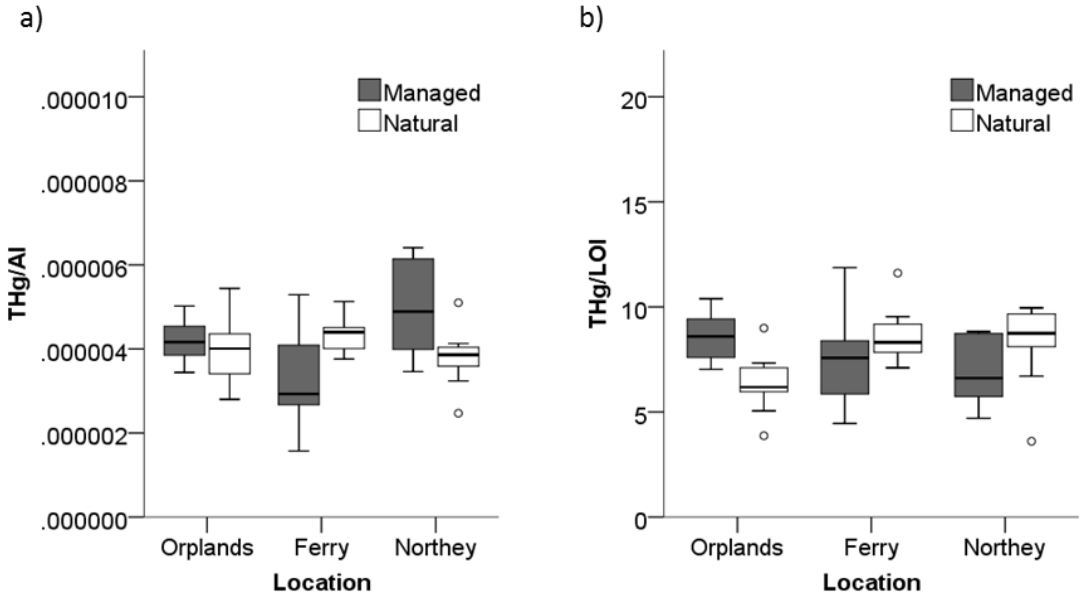

Figure 6.10 (a) THg/Al and (b) THg/LOI in sediment for Orplands, Ferry Lane, and Northey Island. Whiskers indicate the minimum and maximum of all data.

As observed in estuaries elsewhere (Conaway *et al*. 2003, Sunderland *et al*. 2004, Hammerschmidt *et al*. 2004, Hammerschmidt and Fitzgerald 2004, Sunderland *et al*. 2006), the concentration of THg in sediment was correlated to Al (used as a proxy for fine-grained aluminosilicates) and LOI (used as a proxy for organic material present). PCA also indicated that principal component one (PC1), which explained 24% of data variability, was highly loaded by metals including Mg, Cr, Cu, Pb, Ni, K, and to a lesser extent Fe suggesting that metal variability was controlled by the sediment's natural mineralogy. The scores for PC1 correlate significantly with THg. This positive correlation, as well as the positive correlation with LOI, demonstrates that THg in the sediment is likely sorbed to fine-grained material, organic matter and/or organic matter coating fine material. Therefore, factors that control organic matter and fine-grained material distribution also likely control THg distribution. This finding is supported by a positive significant correlation between THg and LOI.

Fe and Mn oxyhydroxides also have a high adsorptive capacity for metal contaminants (Lillebø *et al*. 2010). Hg species preferentially sorb to solid Fe complexes in surface sediments (i.e. Fe oxyhydroxides) rather than Fe monosulphides (i.e. AVS)

(Hammerschmidt *et al*. 2004). This is evident in our data through a significant correlation between sediment THg and sediment Fe concentrations and a non-significant correlation between sediment THg and AVS concentrations (Figure 6.6). Fe is also loaded onto PC1 (indicative of mineralogy). PC1 is significantly correlated with THg and therefore suggests that Fe is also partly responsible for the variability in THg concentrations.

Saltmarshes are low-energy environments and store sediment and contaminants delivered from the overlying water column (Allen and Pye 1992). Adsorption of Hg[II] to organic matter has been identified as an important mechanism that facilitates the transport, as well as the mobility and bioavailability of Hg within the aquatic environment (Bryan and Langston 1992). Mercury is scavenged from the water column by flocculating organic particulate matter (Cossa *et al*. 1988) which either exists as organic particles or as organic coatings on fine-grained inorganic particles (Conaway *et al*. 2003) which is then deposited within the saltmarsh during tidal inundation. Therefore, the source of Hg for these coastal wetlands is likely from the overlying water column.

6.4.3 THg bioavailability

The distribution of THg in porewater was not correlated to sediment THg concentrations (Figure 6.6), suggesting that porewater THg concentrations are not simply controlled by an exchange equilibrium between dissolved and solid phases (Choe *et al*. 2004). Other environmental factors are controlling the partitioning of THg into the aqueous phase.

Sediment THg seems to sorb to Fe oxyhydroxides as discussed in Section 6.4.1. Solubilisation and precipitation reactions involving Fe and Mn have been shown to control the solubility and mobility of THg (Gobeil and Cossa 1993) and hence porewater THg. Here, a positive correlation was observed between porewater THg and Fe[II] (r = 0.422, p , 0.001) indicating that the reduction of Fe oxyhydroxides at the redox boundary results in the partitioning of THg into the aqueous phase. Principal

component two (PC2) was also strongly loaded by porewater Mn and Fe as well as Fe[II] and S^{2-}, suggesting that PC2 represents reducing conditions. Highest Fe[II] concentrations were found at Orplands restored site, where the highest porewater THg concentrations were also found suggesting that microorganisms are utilising Fe oxyhydroxides as other electron acceptors causing the release of associated Hg species, and hence the increase of THg and Fe[II] in porewater. The sediment at Orplands restored site therefore contains a more reducing environment which is facilitating the release of THg into porewater and increasing its bioavailability (Krabbenhoft *et al*. 2005). To enter the aquatic food chain, Hg is transported across the lipid membrane that surrounds the phytoplankton or bacteria. It is mainly uncharged complexes that are able to diffuse through the lipid membrane such as $HgCl_2$, $Hg(HS)_2$, $Hg(OH)_2$ and HgS^0 however which complexes are present in the environment is largely dependent on pH, redox status and chloride concentration (Morel *et al.* 1998).

In previous studies, sediment organic matter content has been shown to have a varied effect on Hg methylation. Organic matter can influence Hg methylation by either promoting MeHg production by providing a substrate for mineralisation and stimulating microbial activity, or by inhibiting MeHg production by complexing with Hg[II] and reducing the amount of bioavailable Hg[II] to methylating bacteria (Sunderland *et al*. 2006, Hammerschmidt *et al*. 2004, Hammerschmidt and Fitzgerald 2004, Ravichandran 2004, Barkay *et al*. 1997). THg partition coefficients do not significantly correlate with LOI data suggesting that other environmental factors are controlling the partitioning of THg into the aqueous phase other than LOI concentrations (i.e. redox status and/or microbial activity). The lack of correlation between MeHg and LOI in this study suggests that the main role of organic matter was as a control on the bioavailability and distribution of THg (reflected by the positive correlation between THg and LOI) and that other environmental factors are controlling MeHg production. Also, LOI is only a proxy for organic matter and does not give an indication of the quality or lability of the organic matter. Therefore, the LOI present

may not correlate strongly with MeHg concentrations if it is of poor quality and not available to SRB and FeRB.

Sulphide concentration is known to have a significant control on the speciation of Hg present in porewater and therefore Hg bioavailability and methylation (Craig and Moreton 1983). Sediment porewaters with low sulphide concentrations (< 340 mg L^{-1}) but equal to or greater than dissolved inorganic Hg (1-10 ng L^{-1}), HgS^0 is predicted to be the dominant Hg[II] complex in coastal marine sediments (see Figure 6.9; Fitzgerald *et al*. 2007). Inorganic Hg must be in the dissolved state (HgS^0) to enter a bacterial cell and be methylated. It is most likely that inorganic Hg enters the bacterial cell by passive diffusion through the cellular membrane as a dissolved, neutrally charged complex (Benoit *et al*. 1999, Benoit *et al*. 2001, Fitzgerald *et al*. 2007). In this study, average sulphide concentrations are always below 0.340 mg L^{-1} except at Orplands restored site where concentrations averaged 0.416 mg L^{-1} suggesting that HgS^0 is the primary Hg[II] species in porewater and that a major fraction of the porewater Hg[II] is bioavailable for uptake and transformation (Fitzgerald *et al.* 2007). Furthermore, sulphide is loaded onto PC2. PC2 is loaded by porewater Fe and Mn, Fe[II], S^{2-}, and sediment AVS and CRS, all variables indicative of reducing conditions. Bivariate plots of PC2 scores show that Orplands restored site has significantly higher values than all other sites suggesting that Orplands restored site contains more reducing sediments.

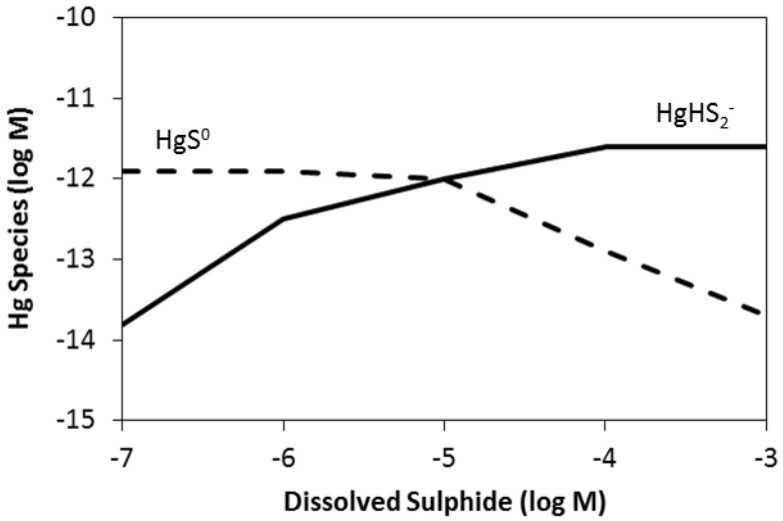

Figure 6.11 Changes in concentrations of HgS^0 and $HgHS_2^-$, the dominant Hg-S complexes in sediment porewaters, as a function of sulphide (taken from Fitzgerald *et al*. (2007) who estimated Hg-S complexes using the solid phase Hg speciation model of Benoit *et al*. (1999)).

A reducing environment will also produce higher sulphide concentrations which also increases the solubility of Hg attributed to competition of sulphide with solid-phase organic matter for Hg[II] (Fitzgerald, Lamborg, and Hammerschmidt 2007). Again, dissolved Hg is more bioavailable and therefore an increase in sulphide (up to ~0.340 mg L^{-1}) will promote Hg methylation. Orplands has more waterlogged and reducing conditions than Ferry Lane or Northey Island (Morris *et al*. 2014) and therefore FeRB and SRB are likely more active in these sediments. Overtime, the elevation increases and the sediments become less waterlogged and hence less anoxic. This is evident in the lower Fe[II] and S^{2-} concentrations at Ferry Lane and Northey Island restored sites. FeRB and SRB are obligate anaerobes and therefore do not respire in oxic conditions, and therefore less MeHg will be produced in these sites (i.e. natural sites, and Ferry Lane and Northey Island restored sites).

6.4.4 Geochemical controls on Hg methylation

Sediment MeHg concentrations are at the high end of values reported for estuarine systems (Mitchell and Gilmour 2008, Choe *et al*. 2004, Conaway *et al*. 2003). A lack of

correlation between THg and MeHg in sediment and porewater suggests that THg concentration is not a key factor controlling MeHg concentrations in this study. Therefore, in these surface sediments, Hg methylation is more likely to depend on Hg[II] bioavailability and the microbial activity that converts Hg[II] to MeHg rather than THg concentrations. Hence wetland processes control MeHg production rather than THg concentrations, a finding that has also been observed in saltmarshes and other systems elsewhere (Yee *et al*. 2005, Grenier *et al*. 2010, Benoit *et al*. 2003, Hsu-Kim *et al*. 2013).

The distribution of MeHg in porewater was significantly correlated to sediment MeHg concentrations suggesting that sediment MeHg concentrations are partly controlled by an exchange equilibrium between dissolved and solid phases. As discussed for THg, solubilisation and precipitation reactions involving Fe and Mn, which depend on redox conditions in the sediment, can also control the solubility and mobility of MeHg. Bloom *et al*. (1999) showed that the partition coefficient for MeHg was lowest at the depth where dissolved Fe concentration was greatest. Strong positive correlations between porewater MeHg and porewater Fe ($r = 0.620$, $p < 0.001$) and porewater Mn ($r = 0.543$, $p < 0.001$) suggests that the mobility of MeHg might also be controlled by dissolution processes involving Fe and Mn oxyhydroxides (i.e. redox status; Choe *et al*. 2004) and not just an exchange equilibrium between dissolved and solid phases. Furthermore, reduced conditions (indicated by increased Fe[II] concentrations) have indicated an increase in porewater THg which is more bioavailable for methylation by sulphate and iron reducing bacteria which could also cause an increase in Hg methylation and hence MeHg concentrations. Therefore, the partitioning of THg between the dissolved and solid phases has some influence on Hg methylation. The partition coefficient (log K_D THg) was negatively correlated to % MeHg in sediment, a proxy used to describe the relative Hg methylation rate (Bloom *et al*. 1999, Choe *et al*. 2004). This shows that there is a positive correlation between the amount of Hg in the dissolved phase and Hg methylation.

Porewater MeHg concentrations and % MeHg were highest in Orplands restored sediments and significantly higher than the natural saltmarsh suggesting that the restored marsh area could be an important source of MeHg into the estuary. The surface sediments at Orplands restored site contained higher Fe[II] and S^{2-} concentrations suggesting that FeRB and SRB are more active in this site. These sediments have also been shown to be more waterlogged than the other sites due to past land use and a lower elevation (Morris *et al*. 2014). Waterlogged sediments are creating reducing conditions as well as the increase in activity of FeRB and SRB. During the process of anaerobic respiration, these microbes are producing MeHg as a by-product of carbon mineralisation. Overtime, as the site becomes less waterlogged (e.g. Ferry Lane and Northey Island), the sediments become less anoxic. FeRB and SRB are unable to respire, indicated by the lower Fe[II], S^{2-}, AVS and CRS concentration at these sites, and hence less MeHg is being produced.

6.4.4.1 Sulphur

As discussed in Section 6.4.3 sulphide concentrations have a control on Hg bioavailability. However, sulphide concentrations in these sediments do not appear to reach levels that create $HgHS_2^-$ complexes which inhibit Hg methylation. Either microbial activity is not great enough to produce inhibitory levels of sulphide or more likely, sulphide is being removed. Sulphide reduction is clearly evident, especially at Orplands restored site, given the increased porewater sulphide concentrations and reduced sulphide within the sediment (AVS and CRS) in the surface sediments. Sediment disturbance can enhance microbial activity by delivering labile organic material to depth but it also removes by-products of sulphate reduction (e.g. sulphide) that have been shown to inhibit SRB methylation (Hammerschmidt and Fitzgerald 2004). Furthermore, sediment disturbance can introduce chemical oxidants (e.g. Fe[III]) that minimise the production and accumulation of sulphide, and promotes dissolved inorganic Hg as HgS^0, the speciation of Hg that is most bioavailable (Benoit *et al*. 1999). Reduced sulphur concentrations can be limited due to oxidation by Fe oxyhydroxides (Canfield *et al*. 1992). Where abundant, Fe oxyhydroxides will therefore mitigate S^{2-} toxicity to SRB. The negative correlation between total Fe concentrations

and reduced sulphur (Figure 6.6) is evidence that this is occurring in the surface sediments at Orplands, Ferry Lane and Northey Island surface sediments.

Previous studies show that MeHg production in saltmarshes is significant but restricted to soil depths where sulphate reduction rates are high but sulphide accumulation rates are low (e.g. Marvin-DiPasquale and Agee 2003, Mitchell and Gilmour 2008). In this study, surface sediments appear to play an important role in MeHg production in restored coastal wetlands. Morris *et al*. (2014) show that for 30 cm sediment cores, Orplands managed realignment site had the lowest MeHg concentrations, however the results shown here indicate that surface sediments at Orplands realignment site have the highest concentrations. This is most likely because the peak in MeHg concentrations at other sites would have been below the surface (2-10 cm depth; Mitchell and Gilmour 2008) whereas because the sediment is waterlogged at Orplands restored site, peak concentration are probably in the surface sediments. However, detailed depth sampling is needed to confirm this hypothesis. Surface sediment MeHg concentrations are arguably more significant given that surface sediment has maximum interaction and flux with the overlying water column during tidal inundation and hence MeHg can be more easily transported to other areas in the estuary.

6.4.4.2 Salinity

Hg methylation has generally been found to be lower in sulphate rich estuarine sediments compared to freshwater sediments because high sulphide levels inhibit Hg methylation rates by either creating complexes that are too large to diffuse over the SRB membrane or by removing inorganic Hg from the solution and precipitating it into the solid phase as HgS (Benoit *et al*. 2003). HgS^0 is the Hg-S complexes are most available to methylating bacteria, and the concentration of sulphide controls the speciation of such complexes (e.g. $HgHS_2^-$, $HgSH^+$, HgS^0) (Benoit *et al*. 1999, Benoit *et al*. 2001). Also, MeHg reacts with H_2S to produce volatile dimethylmercury (Benoit *et al*. 1999) which can leave the aquatic environment to the atmosphere. Principal component four (PC4), was highly loaded by sulphate, chloride, AVS and CRS suggesting that sulphur concentrations (likely sulphate from high concentrations found

in seawater) are controlling 12 % of data variability. PC4 correlates with MeHg porewater data suggesting that sulphate concentration is having a positive influence on MeHg production. Sulphate can promote SRB activity which produces MeHg as well as increased AVS and CRS concentrations (which are both loaded onto PC4). Bivariate plots show that salinity is having a similar influence on samples from all locations although this is to be expected given that they are within close proximity to each other and have been inundated with seawater of similar salinity.

In this study, the sulphate-chloride ratio was negatively and significantly correlated with MeHg concentrations in porewater and porewater % MeHg suggesting that the sulphate-chloride ratio has a negative effect on Hg methylation. The literature suggests that saline water can inhibit Hg methylation through increased levels of sulphide. However, porewater MeHg is positively and significantly correlated with S^{2-} concentrations indicating that S^{2-} concentrations are not inhibiting Hg methylation. Salinity however, also affects Hg-DOM binding because other ions, such as chloride compete with DOM to form metal-ligand complexes. Mercury-chloride complexes are important in high chloride oxic conditions (e.g., seawater) where complexation of Hg with chloride ions form negatively-charged species and inhibit uptake by SRB (Barkay *et al*. 1997, Davis *et al*. 2003); the Hg[II] ion exists primarily as $HgCl_4^{2-}$and $HgCl_3^-$ (Ullrich *et al*. 2001).

6.4.4.3 pH

MeHg concentrations had a significant negative correlation with pH (Figure 6.6), a finding in agreement with other studies (Marvin-DiPasquale and Agee 2003, Winfrey and Rudd 1990). pH was also negatively loaded onto PC2. PC2 was significantly correlated with all MeHg data suggesting that lower pH values were promoting Hg methylation. Enhanced MeHg concentrations are evident at Orplands managed site which also has the lowest pH values. More alkaline sediment, especially at the oxic-anoxic boundary, has been associated with lower MeHg production rates (Marvin-DiPasquale and Agee 2003) and lowering the pH at the aerobic sediment-water interface can result in a two- to threefold increase in the rate of methylmercury

production (Winfrey and Rudd 1990). Miller and Akagi (1979) also found that the release of methylmercury from the sediment surface was enhanced by reduced pH by influencing the sediment-porewater partitioning and availability of THg (Yin *et al*. 1996).

6.4.4.4 Iron

Recent evidence has suggested that dissimilatory SRB may not be the only bacterial group capable of Hg methylation, and that dissimilatory iron-reducing bacteria (dFeRB) are also capable of Hg methylation (Mitchell and Gilmour 2008, Kerin *et al*. 2006, Fleming *et al*. 2006). Jensen *et al*. (2003) found that up to 75 % of organic carbon can be mineralised by dFeRB and that this dominance of Fe reduction was related to a relatively high sediment Fe content in combination with active reworking of the sediment by infauna. In this study, the strong positive correlations between porewater MeHg and porewater Fe, and porewater MeHg and Fe[II] suggests that dFeRB are playing a role in Hg methylation. These correlations are stronger than the association between porewater MeHg and S[II] indicating that dFeRB may be methylating Hg to a greater degree than SRB, a finding that has also been found elsewhere (Fleming *et al*. 2006).

6.5 Conclusions

Overall, sediments in the sampling region contained relatively low levels of THg compared to other studies discussed in the literature (see Table 2.1). THg distribution is likely controlled by the physico-chemical factors that control fine-grained material (including Fe oxyhydroxides), organic matter and/or organic matter coating fine material.

MeHg concentrations are at the high end of values reported for estuarine systems and are highest in the newly restored saltmarsh (Orplands). Orplands restored site contains more waterlogged sediments, which produce more anoxic surface sediments allowing SRB and FeRB to become more active. The lack of correlation between THg and MeHg suggests that Hg methylation depends more on Hg[II] bioavailability and the microbial activity that convert Hg[II] to MeHg and less on THg concentrations.

Solubilisation and precipitation reactions involving Fe and Mn appear to have a significant control over MeHg concentrations. The reduction of Fe oxyhydroxides at the redox boundary, evident here by the increased levels of Fe[II], results in the partitioning of THg into the aqueous phase. Fe and Mn oxyhydroxides also prevent the build-up of toxic by-product of SRB, thereby favouring speciation of dissolved Hg-S complexes as HgS^0, the more bioavailable form.

PCA indicates that redox conditions have the largest correlation with both porewater and sediment MeHg concentrations and that Orplands managed site has lower redox status than the other managed or natural sites, explaining why Orplands managed site has the highest MeHg concentrations. Therefore, in the first few decades following de-embankment restoration could potentially be producing MeHg hotspots, especially in the surface sediments. Coastal areas are not generally considered to be areas of concern for MeHg production because high chloride and sulphide concentrations have been shown to inhibit Hg methylation. However, this research shows that other pathways can also be responsible for Hg methylation (i.e. iron reduction) and therefore coastal sediment can be significant contributors to Hg methylation. This finding could

have wider implications for the wider estuary because the surface sediment is the sediment depth which typically has maximum interaction and flux with the overlying water column during tidal inundation.

Chapter 7: Overview and Conclusions

The overall aim of this thesis was to understand the controls upon Hg dynamics in coastal sites, with specific emphasis on the effects of ecosystem restoration on MeHg production. In order to achieve this aim, a series of investigations were conducted which included two field studies and a laboratory experiment.

The objectives of this study were to:

Objective 1: To assess the spatial variability of THg and MeHg concentrations in natural and de-embanked saltmarsh sediment over a range of spatial scales (Chapter 4).

Objective 2: To examine the association between Hg speciation and indicators of saltmarsh development (Chapter 4).

Objective 3: To explore how changes in these physico-chemical conditions and Hg biogeochemistry have changed with time since de-embankment and hence ecosystem development (Chapter 4).

Objective 4: To explore the timing and magnitude of MeHg flux in terrestrial soils flooded with saline and fresh water (Chapter 5).

Objective 5: To explore the effect of redox status on MeHg concentration in flooded terrestrial soils (Chapter 5).

Objective 6: To explore the association between Hg methylation and environmental parameters in restored coastal wetlands (Chapter 6).

Objective 7: To explore how controls on Hg methylation change with time since de-embankment and hence ecosystem development (Chapter 6).

7.1 Review of the research objectives

This research has produced four key outcomes that will advance knowledge in the fields of coastal wetland biogeochemistry and specifically Hg biogeochemical cycling.

Firstly, there is clear evidence to show that previous land-use has had a significant impact on physico-chemical sediment characteristics (Chapter 4), and as the saltmarsh develops overtime, these characteristics change to reflect more natural saltmarsh conditions. MeHg concentrations in restored coastal wetlands are below natural concentrations for many decades post tidal re-introduction and this reflects the high moisture content of the sediment in newly restored sites. MeHg concentrations in Northey Island (de-embanked more than 100 years ago) were actually higher in the restored site compared to the natural site (approximately 1500 pg g^{-1} higher) indicating that coastal wetland restoration has the potential to produce increased levels of MeHg production than their natural counterpart, an important consideration for coastal managers regarding the future health of the estuary. The detailed vertical profiles of MeHg from paired cores taken from Orplands managed realignment and natural site at equal elevations also indicate that once the elevation of the restored marsh has increased and the sediment is able to drain more efficiently, the restored site produces significantly higher MeHg concentrations (approximately 200 pg g^{-1}) that their natural counterpart. Therefore, although Orplands and Northey Island managed sites as a whole contain less MeHg concentrations than the natural sites, other locations with more equal elevation (i.e. in areas with higher sedimentation rates or with different management practices such as increase elevation before reintroducing tidal inundation) could potentially produce significantly more MeHg.

Secondly, physical sediment properties are less heterogeneous in restored sites at the intermediate scale (15-50 m), which is indicative of lower habitat and topographic heterogeneity. This finding has significant implications for MeHg concentrations and variability in restored coastal wetlands but also for other biogeochemical cycling. Key drivers for coastal restoration are increased de-nitrification, carbon storage and vegetation development, however these ecosystem services may not be taking place at levels comparable to natural saltmarshes due to decreased heterogeneity. Previous studies have indicated that permanently flooded soils produce high MeHg concentration however this is the first study to show that fluctuating saline conditions could produce a pulse of MeHg (a cumulative exposure of 1830 pg g^{-1} over 56 days).

Therefore, coastal restoration may not fulfil its aims and objectives for many decades after de-embankment, an important consideration for coastal managers.

Thirdly, flooding agricultural soils with saline water has been shown to increase THg mobility and MeHg concentrations. Increased MeHg concentrations in saline samples (peak concentration of 284 pg g^{-1}) is attributed to increased SRB activity in saline samples as well as the increased bioavailability of Hg(II) for methylation by SRB. Peaks in MeHg concentrations in fresh water treatments (peak concentration of 163 pg g^{-1}) are attributed to FeRB stimulated by reducing conditions. Redox status has also had a significant effect on MeHg concentrations. Largest peaks in MeHg concentration were evident in fluctuating (oxic/anoxic) conditions. This is an important finding for coastal managers because it indicates that large peaks of MeHg could be produced immediately following tidal inundation and that MeHg could potentially increase overtime as the site develops to a tidal regime more comparable to a natural saltmarsh.

Fourthly, although sediment cores from Orplands managed realignment site showed the lowest concentration of THg (107 ng g^{-1}), surface sediments contained the highest concentration of MeHg (1450 pg g^{-1}) compared to Ferry Lane (1217 pg g^{-1}) and Northey Island (1136 pg g^{-1}) restored sites. Surface sediments have maximum interaction and flux with the overlying water column during tidal inundation so are arguably more important than deeper sediment profiles that does not interact with the overlying water column. Solubilisation and precipitation reactions involving Fe and Mn appear to have a significant control over MeHg concentrations. The reduction of Fe oxyhydroxides at the redox boundary, results in the partitioning of THg into the aqueous phase. Fe and Mn oxyhydroxides also prevent the build-up of toxic by-product of SRB, thereby favouring speciation of dissolved Hg-S complexes as HgS^0, the more bioavailable form. In the first few decades following de-embankment restoration could potentially be producing MeHg hotspots in the surface sediments due to their low elevation and reducing conditions.

This is the first research study on Hg methylation in restored coastal wetlands in the UK and as such provides important information for coastal managers. It is important that the Hg methylation potential is considered before restoration is implemented otherwise hotspot of MeHg could develop.

7.2 Future Research

There are two clear areas of research that would follow on from this PhD research. Firstly, although the laboratory experiments have given a clear indication of the size and magnitude of MeHg production following inundation, they are clearly lacking in simulating environmental conditions, especially in respect to DOC concentrations. For example, MeHg concentrations decrease after a period of ten days which is potentially due to the supply of food source (labile organic carbon) for SRB running out. Repeating these experiments in the field would give a more representable and realistic idea of how MeHg concentrations are likely to change after saline inundation.

Secondly, the sediment profiles taken from Orplands managed realignment and natural site show clear differences in MeHg concentrations from the composited sediment cores also collected from Orplands. There also appears to be differences in the sediment depth where MeHg concentrations peak depending on the location the core was taken from. Surface samples close to the breach (more seaward) have high MeHg concentrations, whereas the samples from the back of the site (more landward) have higher MeHg concentrations at depth. It is important to understand how and when these changes occur as the site develops and also where the peak MeHg concentrations occur in older sites as well as newly restored site (< 1 year) to allow coastal managers to assess how mobile the MeHg is and whether it is likely to have an impact on the surrounding ecosystem. Further research is needed to give a more detailed profile of MeHg concentrations in restored coastal wetlands.

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Appendix 1

Morris, M.A., K.L. Spencer, L.R. Belyea, and B.A. Branfireun. 2014. "Temporal and spatial distributions of sediment mercury in restored coastal saltmarshes." *Marine Chemistry,* 165, 150-159.

Appendix 2

Table S1. Results for mixed effects models for THg and MeHg, as well as physical sediment properties. Significance tests are included for fixed effects only (not significant, ns).

Source of variation	d.f.	Mean square	F	p-value
THg content				
Type (Natural, Restored)	1	0.3967	63.17	<0.001
Type X Site interaction	2	0.0029	0.46	(ns)
Site (Orplands, Ferry, Northey)	2	3.507		
Location (within site)	27	0.0201		
Replication (within location within site)	147	0.0063		
MeHg content				
Type (Natural, Restored)	1	0.3260	4.90	<0.05
Type X Site interaction	2	2.2389	33.65	<0.001
Site (Orplands, Ferry, Northey)	2	0.2355		
Location (within site)	27	0.2112		
Replication (within location within site)	147	0.0665		
Loss on ignition				
Type (Natural, Restored)	1	0.0875	130.72	<0.001
Type X Site interaction	2	0.0279	41.67	<0.001
Site (Orplands, Ferry, Northey)	2	0.0818		
Location (within site)	27	0.0034		
Replication (within location within site)	147	0.0007		
Moisture content				
Type (Natural, Restored)	1	0.1170	95.44	<0.001
Type X Site interaction	2	0.0944	77.04	<0.001
Site (Orplands, Ferry, Northey)	2	0.3125		
Location (within site)	27	0.0116		
Replication (within location within site)	147	0.0012		
Bulk density				
Type (Natural, Restored)	1	0.6763	62.03	<0.001
Type X Site interaction	2	0.3938	36.12	<0.001
Site (Orplands, Ferry, Northey)	2	0.8057		
Location (within site)	27	0.0722		
Replication (within location within site)	147	0.0109		

Appendix 3

Chapter 5: Procedure for fitting model (Ricker Function) to data

1) Offset Ricker Model 1 – Run full model with different coefficient for each treatment.
2) Offset Ricker Model 2 – Coefficient B varies with redox only
3) Offset Ricker Model 3 – Coefficient B varies with salinity only
4) Offset Ricker Model 4 – Coefficient B does not vary with treatment
5) Check AICc

   Offset Ricker Model 1 = 722.2

   Offset Ricker Model 2 = 714.7

   Offset Ricker Model 3 = 728.9

   Offset Ricker Model 4 = 726.0
6) Offset Ricker Model 2 is best model so far – continue with Model 2
7) Offset Ricker Model 5 – Coefficient C varies with redox only
8) Offset Ricker Model 6 – Coefficient C varies with salinity only
9) Offset Ricker Model 7 - Coefficient C does not vary with treatment
10) Check AICc

    Offset Ricker Model 5 = 709.5

    Offset Ricker Model 6 = 712.5

    Offset Ricker Model 7 = *na*
11) Offset Ricker Model 5 is best model so far – continue with Model 5
12) Offset Ricker Model 8 – Coefficient A varies with redox only
13) Offset Ricker Model 9 – Coefficient A varies with salinity only
14) Offset Ricker Model 10 - Coefficient A does not vary with treatment
15) Check AICc

    Offset Ricker Model 8 = 709.6

    Offset Ricker Model 9 = 788.2

    Offset Ricker Model 10 = 842.4

16) Offset Ricker Model 5 is best model – use as final model

Summary of model fitting procedure for the offset Ricker function

Model	log-likelihood	AICc	delta AIC	AIC weight	K (number of estimable parameters)
Offset Ricker Model 1	-335.6	722.2	12.79	0.00	19
Offset Ricker Model 2	-336.9	714.7	5.24	0.03	16
Offset Ricker Model 3	-345.6	728.9	19.45	0.00	15
Offset Ricker Model 4	-345.7	726.0	16.56	0.00	14
Offset Ricker Model 5	-338.9	709.5	0.00	0.45	13
Offset Ricker Model 6	-341.9	712.5	3.10	0.10	12
Offset Ricker Model 7	*na*	*na*	*na*	*na*	*na*
Offset Ricker Model 8	-344.5	709.6	0.12	0.42	9
Offset Ricker Model 9	-385.1	788.2	78.78	0.00	8
Offset Ricker Model 10	-419.1	842.4	132.94	0.00	2

Appendix 4

Raw data from Chapter 4

Depth (cm)	Location	N/M	Sample Date	Sediment					
				THg (ng g^{-1})	MeHg (pg g^{-1})	%MeHg	LOI (%)	Bulk Desity (g cm^{-3})	Moisture Content (%)
27.8	Ferry Lane	MR	07/09/2012	550.32	1020.59	0.19	11.43	0.60	45.41
29.1	Ferry Lane	MR	07/09/2012	561.43	1267.40	0.23	10.98	0.57	44.22
25.4	Ferry Lane	MR	07/09/2012	624.23	870.11	0.14	11.68	0.56	43.61
25.3	Ferry Lane	MR	07/09/2012	414.17	831.74	0.20	10.63	0.70	53.82
27.8	Ferry Lane	MR	07/09/2012	443.12	1237.73	0.28	11.51	0.64	50.09
24.8	Ferry Lane	MR	07/09/2012	458.52	836.41	0.18	11.69	0.73	53.07
27.5	Ferry Lane	MR	07/09/2012	422.36	861.33	0.20	10.51	0.74	54.01
28.2	Ferry Lane	MR	07/09/2012	440.27	1041.96	0.24	11.54	0.65	50.75
28.4	Ferry Lane	MR	07/09/2012	252.06	951.64	0.38	12.10	0.72	52.90
29.5	Ferry Lane	MR	07/09/2012	563.59	1258.44	0.22	11.00	0.62	48.17
26.9	Ferry Lane	MR	07/09/2012	473.97	1007.90	0.21	13.51	0.62	47.83
28.8	Ferry Lane	MR	07/09/2012	629.08	1382.35	0.22	11.28	0.59	45.86
30.0	Ferry Lane	MR	29/10/2012	528.48	920.31	0.17	9.88	0.66	49.42
30.0	Ferry Lane	MR	29/10/2012	488.67	988.41	0.20	9.35	0.66	49.13
30.0	Ferry Lane	MR	29/10/2012	433.16	952.52	0.22	10.00	0.67	49.54
30.0	Ferry Lane	MR	29/10/2012	600.96	1085.64	0.18	8.28	0.67	48.38
30.0	Ferry Lane	MR	29/10/2012	521.27	964.61	0.19	9.45	0.65	47.97
30.0	Ferry Lane	MR	29/10/2012	535.91	1141.80	0.21	9.91	0.65	48.26
30.0	Ferry Lane	MR	29/10/2012	549.06	2142.45	0.39	8.53	0.49	40.23
30.0	Ferry Lane	MR	29/10/2012	519.79	2078.52	0.40	10.61	0.47	38.50
30.0	Ferry Lane	MR	29/10/2012	518.79	1445.65	0.28	11.51	0.49	39.90
30.0	Ferry Lane	MR	29/10/2012	587.98	1041.83	0.18	10.22	0.58	44.84
30.0	Ferry Lane	MR	29/10/2012	741.80	1078.00	0.15	9.82	0.59	45.26

30.0	Ferry Lane	MR	29/10/2012	675.07	1376.17	0.20	9.33	0.60	45.19
30.0	Ferry Lane	MR	29/10/2012	589.64	1329.85	0.23	9.17	0.62	46.76
30.0	Ferry Lane	MR	29/10/2012	511.14	1189.26	0.23	8.49	0.63	46.98
30.0	Ferry Lane	MR	29/10/2012	539.27	1113.34	0.21	8.71	0.63	47.09
30.0	Ferry Lane	MR	29/10/2012	512.97	889.51	0.17	8.48	0.70	50.32
30.0	Ferry Lane	MR	29/10/2012	482.09	896.64	0.19	9.22	0.72	51.01
30.0	Ferry Lane	MR	29/10/2012	523.60	1178.32	0.23	9.05	0.71	50.57
28.0	Northey Island	MR	16/10/2012	290.80	2284.95	0.79	19.02	0.34	29.95
30.0	Northey Island	MR	16/10/2012	337.26	1910.09	0.57	19.58	0.33	30.42
23.0	Northey Island	MR	16/10/2012	403.59	2242.19	0.56	17.41	0.35	30.27
28.7	Northey Island	MR	16/10/2012	313.45	1804.84	0.58	18.22	0.35	30.83
30.0	Northey Island	MR	16/10/2012	408.26	1888.02	0.46	15.94	0.35	30.39
28.3	Northey Island	MR	16/10/2012	359.62	1527.58	0.42	17.55	0.36	30.75
30.0	Northey Island	MR	16/10/2012	331.90	2027.22	0.61	15.96	0.37	32.24
30.0	Northey Island	MR	16/10/2012	339.47	1735.56	0.51	16.60	0.36	32.23
30.0	Northey Island	MR	16/10/2012	363.61	1764.57	0.49	16.93	0.38	32.56
30.0	Northey Island	MR	16/10/2012	373.42	1662.62	0.45	17.26	0.34	30.34
30.0	Northey Island	MR	16/10/2012	359.44	1765.57	0.49	20.56	0.32	28.48
30.0	Northey Island	MR	16/10/2012	339.57	1289.59	0.38	19.50	0.34	29.95
26.4	Northey Island	MR	16/10/2012	433.22	2093.13	0.48	20.78	0.18	33.76
27.2	Northey Island	MR	16/10/2012	385.76	2911.08	0.75	17.06	0.19	35.41
29.1	Northey Island	MR	16/10/2012	397.96	3183.01	0.80	17.81	0.19	34.42
24.1	Northey Island	MR	16/10/2012	371.30	2467.78	0.66	18.47	0.15	28.37
25.2	Northey Island	MR	16/10/2012	342.63	2138.94	0.62	18.31	0.14	27.27
23.3	Northey Island	MR	16/10/2012	346.43	1738.30	0.50	18.94	0.15	29.51

30.0	Northey Island	MR	16/10/2012	164.97	1436.75	0.87	13.64	0.33	51.29
25.2	Northey Island	MR	16/10/2012	158.49	1445.38	0.91	14.62	0.31	48.72
28.1	Northey Island	MR	16/10/2012	197.31	2004.11	1.02	14.09	0.33	51.52
30.0	Northey Island	MR	16/10/2012	426.89	1158.98	0.27	21.90	0.41	35.93
30.0	Northey Island	MR	16/10/2012	383.14	1994.48	0.52	19.06	0.37	33.10
28.7	Northey Island	MR	16/10/2012	275.84	589.31	0.21	19.52	0.48	37.30
29.7	Northey Island	MR	16/10/2012	387.66	1419.58	0.37	17.84	0.32	29.20
29.3	Northey Island	MR	16/10/2012	389.55	1439.55	0.37	17.64	0.30	28.34
30.0	Northey Island	MR	16/10/2012	357.70	1829.88	0.51	21.88	0.14	27.07
24.8	Northey Island	MR	16/10/2012	241.19	2288.56	0.95	13.80	0.63	47.30
26.0	Northey Island	MR	16/10/2012	175.34	1246.99	0.71	10.01	0.64	48.32
24.7	Northey Island	MR	16/10/2012	194.05	1495.71	0.77	14.18	0.65	48.60
30.0	Orplands	MR	24/09/2012	151.40	757.93	0.50	10.06	0.76	55.53
29.5	Orplands	MR	24/09/2012	206.75	867.52	0.42	13.51	0.71	56.15
28.1	Orplands	MR	24/09/2012	208.87	597.51	0.29	11.70	0.72	55.75
28.3	Orplands	MR	24/09/2012	197.77	1138.51	0.58	12.05	0.68	50.55
30.0	Orplands	MR	24/09/2012	168.23	950.20	0.56	11.95	0.70	55.01
28.9	Orplands	MR	24/09/2012	176.21	1228.04	0.70	12.85	0.72	55.20
30.0	Orplands	MR	24/09/2012	195.33	2006.53	1.03	11.52	0.78	53.64
30.0	Orplands	MR	24/09/2012	174.40	26.50	0.02	12.03	0.77	54.81
28.6	Orplands	MR	24/09/2012	198.80	106.63	0.05	11.55	0.72	51.98
27.9	Orplands	MR	24/09/2012	185.81	0.00	0.00	12.49	0.67	50.15
29.5	Orplands	MR	24/09/2012	198.79	16.38	0.01	12.70	0.67	49.95
28.6	Orplands	MR	24/09/2012	170.50	253.16	0.15	12.75	0.72	55.57
28.0	Orplands	MR	24/09/2012	132.95	814.66	0.61	9.37	0.85	62.25

28.6	Orplands	MR	24/09/2012	139.35	1144.85	0.82	8.33	0.83	58.81
25.1	Orplands	MR	24/09/2012	127.05	1326.28	1.04	7.92	0.88	59.81
28.7	Orplands	MR	24/09/2012	161.06	1900.87	1.18	8.03	0.87	58.03
29.0	Orplands	MR	24/09/2012	148.52	1563.74	1.05	8.72	0.84	58.85
28.9	Orplands	MR	24/09/2012	151.48	1946.95	1.29	8.27	0.89	61.50
29.7	Orplands	MR	24/09/2012	202.88	885.70	0.44	11.08	0.31	49.59
30.0	Orplands	MR	24/09/2012	227.04	159.69	0.07	10.84	0.30	48.72
28.5	Orplands	MR	24/09/2012	234.74	158.56	0.07	11.06	0.28	46.71
27.5	Orplands	MR	24/09/2012	204.48	995.99	0.49	10.39	0.74	55.34
29.3	Orplands	MR	24/09/2012	210.51	898.09	0.43	10.88	0.77	56.42
29.7	Orplands	MR	24/09/2012	178.34	992.84	0.56	11.88	0.71	55.52
24.8	Orplands	MR	24/09/2012	111.94	879.56	0.79	9.56	0.46	64.36
29.4	Orplands	MR	24/09/2012	123.28	554.30	0.45	9.95	0.44	62.24
26.6	Orplands	MR	24/09/2012	124.58	589.06	0.47	10.91	0.88	61.05
25.0	Orplands	MR	24/09/2012	114.48	460.26	0.40	9.65	0.87	58.22
25.8	Orplands	MR	24/09/2012	141.10	752.95	0.53	10.55	0.92	63.15
27.2	Orplands	MR	24/09/2012	141.14	519.86	0.37	8.81	0.83	57.29
30.4	Ferry Lane	N	07/09/2012	857.51	725.20	0.08	17.31	0.48	39.71
29.5	Ferry Lane	N	07/09/2012	843.26	777.57	0.09	18.80	0.48	39.26
30.1	Ferry Lane	N	07/09/2012	1018.43	893.25	0.09	18.43	0.46	38.68
28.6	Ferry Lane	N	07/09/2012	641.64	1035.93	0.16	15.54	0.53	42.97
29.8	Ferry Lane	N	07/09/2012	668.18	1026.08	0.15	14.39	0.55	43.44
30.6	Ferry Lane	N	07/09/2012	835.16	839.01	0.10	14.64	0.55	43.58
28.5	Ferry Lane	N	07/09/2012	804.92	803.79	0.10	15.96	0.65	49.11
30.0	Ferry Lane	N	07/09/2012	1264.96	995.06	0.08	13.37	0.65	48.87

30.0	Ferry Lane	N	07/09/2012	448.19	1060.11	0.24	13.08	0.60	47.42
29.1	Ferry Lane	N	07/09/2012	653.59	1464.70	0.22	15.05	0.21	41.49
25.7	Ferry Lane	N	07/09/2012	493.37	1575.12	0.32	16.96	0.24	40.63
26.6	Ferry Lane	N	07/09/2012	570.83	1589.24	0.28	16.65	0.23	40.07
27.1	Ferry Lane	N	07/09/2012	496.83	1443.85	0.29	16.26	0.24	41.13
28.9	Ferry Lane	N	07/09/2012	468.84	1877.14	0.40	14.35	0.24	41.48
26.2	Ferry Lane	N	07/09/2012	432.39	1179.61	0.27	14.64	0.22	41.74
29.4	Ferry Lane	N	07/09/2012	684.82	914.87	0.13	13.82	0.58	46.08
28.3	Ferry Lane	N	07/09/2012	495.72	730.77	0.15	11.89	0.67	47.83
27.7	Ferry Lane	N	07/09/2012	533.78	728.12	0.14	13.36	0.27	48.42
28.8	Ferry Lane	N	07/09/2012	563.72	1391.17	0.25	14.23	0.51	40.18
31.7	Ferry Lane	N	07/09/2012	561.90	2890.28	0.51	16.52	0.50	41.31
28.8	Ferry Lane	N	07/09/2012	517.78	1613.32	0.31	15.22	0.48	38.96
32.0	Ferry Lane	N	07/09/2012	616.95	1012.59	0.16	14.59	0.48	41.07
30	Ferry Lane	N	07/09/2012	737.57	1680.04	0.23	14.17	0.59	46.21
30	Ferry Lane	N	07/09/2012	636.40	1854.14	0.29	15.49	0.55	43.97
23.7	Ferry Lane	N	07/09/2012	544.35	1857.84	0.34	13.89	0.26	44.77
26.4	Ferry Lane	N	07/09/2012	559.38	1905.74	0.34	13.01	0.26	42.56
28.1	Ferry Lane	N	07/09/2012	529.11	1552.70	0.29	14.61	0.24	42.04
28.6	Ferry Lane	N	07/09/2012	553.16	1721.33	0.31	17.16	0.70	51.16
26.3	Ferry Lane	N	07/09/2012	528.01	2285.73	0.43	12.34	0.65	49.31
22.4	Ferry Lane	N	07/09/2012	572.37	1975.62	0.35	10.41	0.75	54.98
30	Northey Island	N	16/10/2012	434.33	2389.05	0.55	15.02	0.45	37.86
30	Northey Island	N	16/10/2012	430.85	1250.69	0.29	15.65	0.47	39.07
30	Northey Island	N	16/10/2012	453.04	1354.05	0.30	15.93	0.47	40.23

30	Northey Island	N	16/10/2012	426.50	1248.61	0.29	14.64	0.38	34.58
30	Northey Island	N	16/10/2012	351.94	1638.26	0.47	18.68	0.38	34.36
29.2	Northey Island	N	16/10/2012	431.50	1764.81	0.41	15.06	0.40	34.64
28.6	Northey Island	N	16/10/2012	357.86	1697.27	0.47	20.70	0.37	31.43
28.2	Northey Island	N	16/10/2012	388.09	1059.85	0.27	17.04	0.36	31.20
28.4	Northey Island	N	16/10/2012	341.08	653.11	0.19	21.54	0.35	30.70
30	Northey Island	N	25/10/2012	411.34	1365.54	0.33	21.65	0.35	30.07
30	Northey Island	N	25/10/2012	385.78	1575.61	0.41	20.88	0.35	29.87
29.6	Northey Island	N	25/10/2012	481.77	2185.85	0.45	19.94	0.36	30.70
30	Northey Island	N	25/10/2012	434.01	2162.84	0.50	20.83	0.35	29.66
29.5	Northey Island	N	25/10/2012	379.84	2035.80	0.54	20.88	0.35	30.29
30	Northey Island	N	25/10/2012	400.78	2808.77	0.70	20.23	0.34	29.71
27.7	Northey Island	N	25/10/2012	429.39	1891.83	0.44	18.43	0.20	35.41
26.0	Northey Island	N	25/10/2012	433.39	1242.68	0.29	17.12	0.19	34.31
25.6	Northey Island	N	25/10/2012	443.70	1466.96	0.33	17.97	0.19	34.05
30	Northey Island	N	25/10/2012	387.78	232.33	0.06	21.03	0.18	32.86
30	Northey Island	N	25/10/2012	382.18	725.20	0.19	18.70	0.18	33.48
28.7	Northey Island	N	25/10/2012	373.93	490.91	0.13	20.06	0.18	32.90
29.2	Northey Island	N	25/10/2012	383.30	589.98	0.15	16.78	0.52	40.76
27.3	Northey Island	N	25/10/2012	306.73	505.01	0.16	18.75	0.48	38.26
28.8	Northey Island	N	25/10/2012	304.24	0.31	0.00	17.23	0.46	38.21
30.0	Northey Island	N	25/10/2012	457.73	749.88	0.16	12.17	0.63	46.56
27.8	Northey Island	N	25/10/2012	424.81	347.96	0.08	11.65	0.29	46.47
30.0	Northey Island	N	25/10/2012	475.90	770.30	0.16	11.09	0.63	46.45
30.0	Northey Island	N	25/10/2012	436.00	618.01	0.14	11.07	0.67	49.27

30	Northey Island	N	25/10/2012	415.88	878.07	0.21	12.08	0.66	48.78
30	Northey Island	N	25/10/2012	452.85	333.66	0.07	10.50	0.66	48.98
28.9	Orplands	N	24/09/2012	211.99	785.92	0.37	14.62	0.23	40.09
30.0	Orplands	N	24/09/2012	209.96	570.19	0.27	14.29	0.24	39.81
30.0	Orplands	N	24/09/2012	206.22	389.39	0.19	11.39	0.26	40.84
30.0	Orplands	N	24/09/2012	231.65	1279.29	0.55	13.19	0.55	41.67
30.0	Orplands	N	24/09/2012	172.64	673.67	0.39	11.85	0.28	47.88
30.0	Orplands	N	24/09/2012	185.51	2422.02	1.31	12.43	0.26	42.99
28.5	Orplands	N	18/10/2012	271.07	765.69	0.28	12.10	0.56	43.86
29.4	Orplands	N	18/10/2012	262.18	825.95	0.32	12.05	0.58	44.69
29.7	Orplands	N	18/10/2012	232.67	1050.35	0.45	12.64	0.59	45.19
27.1	Orplands	N	18/10/2012	228.46	2856.90	1.25	15.52	0.45	37.25
29.2	Orplands	N	18/10/2012	214.67	2343.27	1.09	15.94	0.45	37.28
30.0	Orplands	N	18/10/2012	222.71	3898.74	1.75	14.14	0.46	38.18
30.0	Orplands	N	18/10/2012	227.44	1985.15	0.87	16.17	0.53	42.78
30.0	Orplands	N	18/10/2012	253.10	1619.71	0.64	16.42	0.56	43.37
29.7	Orplands	N	18/10/2012	287.75	1342.74	0.47	16.05	0.53	42.69
28.0	Orplands	N	18/10/2012	159.89	689.17	0.43	12.37	0.27	43.73
28.1	Orplands	N	18/10/2012	150.49	813.71	0.54	12.63	0.27	43.77
27.9	Orplands	N	18/10/2012	165.36	1017.91	0.62	15.25	0.28	44.12
30.0	Orplands	N	18/10/2012	206.22	2773.72	1.35	18.53	0.50	42.05
29.0	Orplands	N	18/10/2012	191.66	3463.54	1.81	19.48	0.48	40.76
29.1	Orplands	N	18/10/2012	203.58	3307.58	1.62	19.52	0.46	39.94
30.0	Orplands	N	18/10/2012	174.50	3708.99	2.13	16.91	0.50	40.78
30.0	Orplands	N	18/10/2012	219.71	3152.98	1.44	18.01	0.48	40.65

30.0	Orplands	N	18/10/2012	223.81	2451.15	1.10	17.09	0.49	41.37
30.0	Orplands	N	18/10/2012	180.82	1462.84	0.81	16.33	0.58	45.92
30.0	Orplands	N	18/10/2012	206.93	1443.63	0.70	18.35	0.59	46.48
30.0	Orplands	N	18/10/2012	237.40	905.92	0.38	16.15	0.58	46.10
30.0	Orplands	N	18/10/2012	198.89	2217.61	1.12	17.33	0.54	43.21
30.0	Orplands	N	18/10/2012	192.92	2651.40	1.37	15.74	0.55	45.40
29.8	Orplands	N	18/10/2012	187.83	2089.62	1.11	16.60	0.53	42.63

Appendix 5

Raw data from Chapter 5

ID	Day	%MeHg	MeHg (pg g^{-1})	THg (ng g^{-1})	Nitrogen (%)	Carbon (%)
OF1	1	0.15	97.90	64.47	0.17	2.12
OF2	2	0.18	100.07	63.11	0.16	2.02
OF3	3	0.14	86.90	65.38	0.15	2.07
OF4	4	0.16	96.44	64.44	0.12	2.01
OF5	5	0.12	81.84	71.13	0.16	2.02
OF6	6	0.13	79.35	60.26	0.10	2.01
OF7	7	0.12	78.37	64.05	0.12	2.12
OF8	14	0.16	100.82	62.85	0.12	2.10
OF9	21	0.13	89.05	64.33	0.11	1.70
OF10	28	0.11	89.13	71.17	0.14	2.04
OF11	42	0.13	79.89	64.08	0.16	2.03
OF12	56	0.17	99.35	65.53	0.13	1.98
OS1	1	0.23	157.73	64.06	0.12	1.90
OS2	2	0.17	99.46	59.83	0.09	1.86
OS3	3	0.16	108.71	65.29	0.11	1.96
OS4	4	0.17	110.87	65.36	0.10	1.99
OS5	5	0.14	96.03	65.18	0.13	1.83
OS6	6	0.20	125.10	64.07	0.12	2.04
OS7	7	0.17	113.48	63.11	0.10	1.88
OS8	14	0.10	63.98	64.61	0.09	1.79
OS9	21	0.12	74.64	59.66	0.12	1.76
OS10	28	0.11	64.99	63.36	0.11	1.88
OS11	42	0.09	56.29	61.10	0.10	1.81
OS12	56	0.07	59.08	63.94	0.13	1.84
AF1	1	0.24	166.47	64.48	0.39	2.06
AF2	2	0.24	148.60	62.63	0.49	2.15
AF3	3	0.20	149.92	70.98	0.45	1.99
AF4	4	0.23	148.00	67.49	0.41	2.02
AF5	5	0.23	133.70	64.89	0.41	1.93
AF6	6	0.18	124.47	67.02	0.32	1.92
AF7	7	0.23	152.48	65.92	0.37	1.81
AF8	14	0.22	127.45	65.02	0.39	1.98
AF9	21	0.18	125.79	64.89	0.37	1.99
AF10	28	0.15	105.88	65.84	0.34	2.00
AF11	42	0.14	91.51	62.38	0.36	1.97
AF12	56	0.14	91.85	61.91	0.45	1.87

AS1	1	0.31	195.24	64.87	0.28	1.73
AS2	2	0.29	183.20	62.19	0.23	2.28
AS3	3	0.32	191.28	61.33	0.23	1.98
AS4	4	0.27	181.42	60.68	0.20	1.75
AS5	5	0.23	143.95	62.26	0.16	1.92
AS6	6	0.17	108.69	60.85	0.20	1.84
AS7	7	0.23	140.20	58.83	0.17	1.81
AS8	14	0.16	96.86	60.82	0.19	1.93
AS9	21	0.16	90.10	56.27	0.16	1.89
AS10	28	0.17	101.95	60.57	0.17	2.17
AS11	42	0.15	84.94	62.05	0.18	1.96
AS12	56	0.10	66.70	61.44	0.17	1.92
FF1	1	0.18	131.05	65.45	0.14	2.13
FF2	2	0.22	138.18	65.68	0.14	2.10
FF3	3	0.23	146.01	65.38	0.11	1.96
FF4	4	0.22	135.68	63.71	0.14	2.05
FF5	5	0.18	122.74	64.50	0.12	2.03
FF6	6	0.21	121.73	63.96	0.12	1.97
FF7	7	0.23	152.03	64.11	0.14	1.93
FF8	14	0.16	105.80	65.51	0.13	1.93
FF9	21	0.20	130.18	66.40	0.13	1.88
FF10	28	0.15	97.53	63.50	0.14	2.10
FF11	42	0.16	100.01	65.89	0.13	2.04
FF12	56	0.15	93.16	62.19	0.16	2.00
FS1	1	0.36	219.26	61.02	0.12	1.96
FS2	2	0.44	268.41	60.02	0.30	1.84
FS3	3	0.48	286.87	61.89	0.28	2.07
FS4	4	0.46	277.50	61.84	0.30	1.93
FS5	5	0.39	248.40	65.93	0.30	1.95
FS6	6	0.37	237.25	60.16	0.30	1.93
FS7	7	0.45	287.28	62.13	0.31	1.98
FS8	14	0.27	166.99	61.27	0.29	1.74
FS9	21	0.15	122.34	72.02	0.31	1.89
FS10	28	0.20	123.67	61.11	0.32	1.97
FS11	42	0.12	76.54	59.97	0.33	1.93
FS12	56	0.15	74.68	61.56	0.31	1.79

Appendix 6

Raw data from Chapter 6

Sample	Location	N/M	Sample Date	Sediment							
				THg (ng g^{-1})	MeHg (ng g^{-1})	Hg(II) (ng g^{-1})	%MeHg	LOI (%)	pH	AVS (umol g^{-1})	CRS (umol g^{-1})
OM1	Orplands	Managed	14/03/2013	106.10	1.42	104.68	1.34	14.72	7.04	1.89	16.37
OM2	Orplands	Managed	14/03/2013	129.32	1.49	127.83	1.15	14.78	7.01	2.36	24.81
OM3	Orplands	Managed	14/03/2013	126.33	1.79	124.54	1.42	13.40	7.30	2.84	35.46
OM4	Orplands	Managed	14/03/2013	120.58	0.80	119.79	0.66	11.60	7.16	1.47	34.23
OM5	Orplands	Managed	14/03/2013	120.90	1.50	119.40	1.24	15.92	7.17	2.98	43.74
OM6	Orplands	Managed	14/03/2013	115.12	1.05	114.07	0.91	16.37	7.20	3.11	36.87
OM7	Orplands	Managed	14/03/2013	128.05	0.55	127.50	0.43	14.23	7.26	2.04	30.60
OM8	Orplands	Managed	14/03/2013	146.88	2.60	144.28	1.77	15.04	7.09	4.07	28.01
OM9	Orplands	Managed	14/03/2013	117.98	1.91	116.07	1.62	13.99	6.88	2.93	29.33
OM10	Orplands	Managed	14/03/2013	107.20	1.39	105.81	1.29	13.79	7.17	3.60	30.31
ON1	Orplands	Natural	12/03/2013	87.48	0.43	87.05	0.49	14.09	7.51	1.78	14.23
ON2	Orplands	Natural	12/03/2013	69.76	0.55	69.21	0.79	11.61	7.49	1.89	27.05
ON3	Orplands	Natural	12/03/2013	105.54	0.64	104.91	0.60	14.86	7.24	2.45	19.94
ON4	Orplands	Natural	12/03/2013	99.03	0.17	98.86	0.17	11.02	7.32	0.88	13.22
ON5	Orplands	Natural	12/03/2013	96.07	1.21	94.87	1.26	15.62	7.23	1.78	13.97
ON6	Orplands	Natural	12/03/2013	104.00	0.71	103.29	0.68	14.18	7.17	1.34	11.90
ON7	Orplands	Natural	12/03/2013	104.32	3.07	101.25	2.94	26.95	7.47	4.22	29.71
ON8	Orplands	Natural	12/03/2013	103.04	0.94	102.10	0.91	17.28	7.04	1.58	14.08
ON9	Orplands	Natural	12/03/2013	88.83	1.02	87.81	1.15	17.58	7.32	1.24	7.75
ON10	Orplands	Natural	12/03/2013	94.68	1.13	93.54	1.20	14.57	7.28	1.27	11.08

FM1	Ferry	Managed	14/04/2013	122.92	0.81	122.11	0.66	13.74	7.46	0.82	9.61
FM2	Ferry	Managed	14/04/2013	101.30	0.82	100.48	0.81	12.07	7.46	0.94	8.31
FM3	Ferry	Managed	14/04/2013	107.22	1.11	106.11	1.04	14.69	7.37	1.24	6.21
FM4	Ferry	Managed	14/04/2013	149.17	0.78	148.40	0.52	12.56	7.72	0.61	4.89
FM5	Ferry	Managed	14/04/2013	107.34	0.77	106.57	0.71	13.67	7.59	1.14	7.59
FM6	Ferry	Managed	14/04/2013	100.34	0.64	99.70	0.64	16.18	7.65	0.78	7.81
FM7	Ferry	Managed	14/04/2013	61.15	7.46	53.69	12.19	11.50	7.21	2.50	10.57
FM8	Ferry	Managed	14/04/2013	88.67	0.49	88.18	0.55	15.13	7.39	1.31	6.57
FM9	Ferry	Managed	14/04/2013	152.29	0.71	151.57	0.47	18.42	7.20	1.90	24.91
FM10	Ferry	Managed	14/04/2013	46.00	0.59	45.41	1.28	10.33	7.65	1.30	9.47
FN1	Ferry	Natural	14/04/2013	110.97	1.49	109.48	1.34	30.16	7.01	1.31	10.94
FN2	Ferry	Natural	14/04/2013	133.43	0.81	132.62	0.61	16.22	7.71	0.70	3.98
FN3	Ferry	Natural	14/04/2013	138.77	0.84	137.92	0.61	19.54	7.58	0.96	4.10
FN4	Ferry	Natural	14/04/2013	157.10	1.01	156.09	0.64	17.10	7.46	0.84	11.24
FN5	Ferry	Natural	14/04/2013	150.54	0.67	149.86	0.45	17.93	7.44	1.79	11.92
FN6	Ferry	Natural	14/04/2013	138.24	0.73	137.51	0.53	16.00	7.53	0.93	6.17
FN7	Ferry	Natural	14/04/2013	150.02	0.53	149.49	0.35	19.12	7.35	1.22	10.81
FN8	Ferry	Natural	14/04/2013	150.80	1.67	149.13	1.11	18.46	7.21	2.57	6.77
FN9	Ferry	Natural	14/04/2013	158.59	1.80	156.79	1.13	13.66	7.08	1.26	9.42
FN10	Ferry	Natural	14/04/2013	200.45	1.26	199.19	0.63	21.02	7.26	2.49	12.43
NM1	Northey	Managed	03/04/2013	106.49	1.28	105.21	1.20	22.65	7.79	1.49	8.52
NM2	Northey	Managed	03/04/2013	102.34	1.53	100.80	1.50	17.83	7.79	0.95	5.54
NM3	Northey	Managed	03/04/2013	153.29	0.76	152.53	0.49	17.56	7.68	0.80	5.71
NM4	Northey	Managed	03/04/2013	117.79	0.60	117.18	0.51	19.22	7.45	0.62	7.41
NM5	Northey	Managed	03/04/2013	147.37	1.31	146.07	0.89	16.74	7.44	0.97	5.55

NM6	Northey	Managed	03/04/2013	107.04	0.94	106.09	0.88	20.28	7.22	0.74	18.49
NM7	Northey	Managed	03/04/2013	159.39	0.75	158.64	0.47	24.62	7.48	0.72	12.87
NM8	Northey	Managed	03/04/2013	168.32	0.68	167.64	0.40	19.07	7.20	0.84	6.00
NM9	Northey	Managed	03/04/2013	174.82	2.56	172.26	1.46	25.38	7.41	3.21	45.86
NM10	Northey	Managed	03/04/2013	142.60	0.95	141.65	0.67	21.12	7.31	0.75	5.98
NN1	Northey	Natural	03/04/2013	84.68	0.71	83.97	0.83	23.48	7.18	0.93	10.90
NN2	Northey	Natural	03/04/2013	132.30	1.42	130.89	1.07	15.00	7.19	0.32	3.24
NN3	Northey	Natural	03/04/2013	151.87	1.04	150.83	0.69	18.65	7.17	0.96	6.86
NN4	Northey	Natural	03/04/2013	159.11	0.75	158.37	0.47	15.99	7.31	1.00	7.52
NN5	Northey	Natural	03/04/2013	172.53	0.77	171.76	0.45	17.85	7.28	1.20	13.72
NN6	Northey	Natural	03/04/2013	142.74	1.12	141.62	0.78	21.29	7.46	1.86	16.54
NN7	Northey	Natural	03/04/2013	151.71	1.00	150.71	0.66	18.70	7.38	0.70	5.60
NN8	Northey	Natural	03/04/2013	143.96	1.30	142.66	0.90	16.61	7.39	0.74	10.57
NN9	Northey	Natural	03/04/2013	201.14	1.25	199.90	0.62	10.28	7.52	0.35	9.76
NN10	Northey	Natural	03/04/2013	169.26	0.94	168.32	0.55	18.14	7.34	0.95	4.06

Sample	Sediment (mg kg^{-1})											
	TFe	Mn	Al	Ca	Cd	Co	Cr	Cu	K	Mg	Ni	Pb
OM1	38450	951	27594	34453	6.60	18.90	58.45	20.88	8005	11547	40.98	42.42
OM2	42603	591	31624	36865	7.79	19.34	63.76	22.19	8871	12030	43.36	42.59
OM3	39374	383	32280	28115	6.09	18.25	63.22	23.59	9019	11642	41.01	41.85
OM4	49510	608	35007	33840	7.61	22.20	67.73	23.50	10214	13190	45.31	43.61
OM5	41513	664	28327	25870	6.08	20.61	57.99	19.82	8143	10764	41.38	36.88
OM6	38075	495	25370	21269	6.38	19.29	52.94	20.68	7873	10948	40.55	36.55
OM7	39503	480	25485	36633	4.33	18.33	56.43	22.88	7455	11591	40.80	43.25
OM8	39639	493	29775	33015	6.58	18.89	59.93	22.28	8585	11535	40.81	40.74
OM9	37240	572	27803	32814	6.03	19.79	58.18	21.67	8344	11204	42.49	38.63
OM10	39838	1118	30722	19529	8.97	21.43	60.60	20.20	9002	11441	44.30	36.44
ON1	40374	431	25616	7891	8.86	18.26	56.13	24.15	7756	10685	43.61	39.26
ON2	43100	851	24887	10171	8.34	24.89	56.82	22.22	7142	9967	46.99	38.22
ON3	47852	727	30917	9625	7.66	22.99	64.06	26.29	8876	12790	49.67	42.74
ON4	37833	304	29114	5519	6.85	18.25	60.81	32.91	8392	10559	44.47	37.73
ON5	43582	530	20901	15652	7.22	19.60	49.89	23.54	6915	11609	44.28	44.06
ON6	44246	494	24923	8452	7.92	20.12	58.14	23.13	7616	10861	44.11	43.21
ON7	41978	680	19179	5814	5.91	21.80	47.23	21.39	6996	10772	43.00	36.21
ON8	41769	822	23652	8306	8.98	23.72	54.30	22.87	7835	11977	47.05	41.40
ON9	43210	1096	23063	7159	9.62	24.72	57.27	24.32	7054	10829	48.56	45.57
ON10	40998	776	21892	7541	8.28	22.25	53.36	24.35	7155	10931	45.04	48.87

FM1	47370	718	41366	39711	5.69	24.24	80.55	34.20	11169	14550	51.96	59.34
FM2	47293	803	37919	38629	8.08	23.32	76.32	34.29	10323	13850	50.74	57.56
FM3	47836	698	37791	38054	6.59	22.40	76.80	33.98	10230	14099	50.20	60.33
FM4	47315	726	33666	37189	5.10	23.63	72.78	32.36	9168	13660	49.71	59.93
FM5	44998	679	26258	34670	4.25	19.97	57.77	24.78	7659	12852	41.72	52.34
FM6	45744	691	27588	36305	5.27	20.68	60.95	32.88	8080	12975	44.54	58.35
FM7	45800	711	32444	30353	6.84	23.60	70.06	29.78	9809	13603	52.09	63.95
FM8	44932	911	30676	29100	7.03	24.68	65.93	31.96	9092	13857	49.51	70.05
FM9	40330	900	28804	23851	9.96	22.68	62.37	30.17	8565	12560	49.24	61.48
FM10	47509	814	29230	36712	9.16	23.20	66.29	34.32	8513	13474	47.96	68.55
FN1	36328	1388	25539	17823	7.72	25.09	54.59	28.93	8350	11864	44.68	53.23
FN2	44542	1225	29734	34009	7.51	25.79	65.64	30.34	8769	13261	49.96	60.29
FN3	43076	1021	36938	26723	7.20	24.64	72.63	28.32	9988	13350	51.70	53.12
FN4	81786	6035	35275	8278	27.31	73.15	68.50	27.57	10107	13027	62.78	56.85
FN5	42438	905	36586	16855	10.12	24.28	74.47	32.19	10123	13510	54.11	66.90
FN6	42356	869	36685	31081	6.96	23.09	70.95	37.39	10177	13436	48.46	57.29
FN7	43888	1391	29240	31795	11.72	26.02	64.01	31.25	8834	13496	51.93	61.99
FN8	49519	1570	33454	15828	15.51	31.32	69.97	31.84	10015	13771	55.81	61.68
FN9	46897	649	39498	37507	6.27	22.57	76.17	33.81	11152	13916	51.17	57.33
FN10	47775	1170	39511	36662	9.45	24.64	77.23	35.09	10890	13885	52.86	60.86
NM1	41975	628	24925	7434	7.26	18.88	56.73	25.97	8177	12299	45.85	47.40
NM2	41625	651	25907	18517	7.47	18.48	54.73	22.77	8158	11808	41.11	40.98
NM3	46249	446	31031	9750	8.06	18.47	63.43	27.77	8794	12469	43.67	47.30
NM4	42542	450	29519	9438	6.82	18.52	61.32	27.93	8345	12146	44.78	46.01
NM5	42143	320	30438	9222	6.55	16.19	62.02	28.79	8748	11904	43.04	48.01

NM6	42960	754	30922	15264	8.99	20.85	62.73	24.88	9119	12297	46.71	44.17
NM7	41001	413	24880	6204	7.91	18.08	57.35	28.06	7754	11659	45.93	54.39
NM8	38750	279	27391	7795	5.15	15.44	59.31	30.37	8037	11344	42.90	48.92
NM9	43311	295	27534	5144	6.80	15.17	58.44	26.10	8491	10773	40.37	59.83
NM10	39527	269	27082	9122	7.07	15.49	59.46	26.80	8156	12005	43.46	48.71
NN1	49340	1385	34228	9664	13.41	26.31	71.13	30.83	9739	13381	54.58	54.38
NN2	51483	1607	40792	32593	10.19	24.16	73.26	27.94	10736	13616	49.14	52.06
NN3	49520	596	39561	25448	7.02	21.36	73.63	31.48	10687	13617	49.10	62.05
NN4	48400	434	40988	14717	7.90	19.19	78.04	33.35	11090	14022	50.89	59.29
NN5	58392	511	42682	14685	8.81	21.73	82.47	35.58	11383	14163	55.20	70.88
NN6	51703	558	39609	13284	9.03	21.84	75.10	31.88	11058	14199	52.33	52.71
NN7	57667	676	29767	22992	7.86	21.34	60.63	27.33	8760	12931	44.76	49.56
NN8	51067	659	40045	24807	8.58	22.24	74.55	29.45	10891	14046	49.53	51.99
NN9	58433	588	48657	9396	11.14	23.07	90.93	35.98	12527	14745	54.14	66.23
NN10	53813	600	42353	14917	8.32	20.56	77.20	29.83	11440	14402	50.17	59.45

Sample	Porewater										
	THg (ng L^{-1})	MeHg (ng L^{-1})	Hg(II) (ng L^{-1})	%MeHg	TFe (mg L^{-1})	Fe^{2+} (mg L^{-1})	Mn (mg L^{-1})	H_2S (mg L^{-1})	SO_4^{2-} (mg L^{-1})	Cl^- (mg L^{-1})	SO_4^{2-} /Cl^- (mg L^{-1})
OM1	8.53	0.19	8.35	2.19	0.16	0.03	4.55	0.55	1932.54	13868.93	0.1393
OM2	10.32	1.82	8.50	17.61	3.63	2.12	6.49	0.47	2160.11	15156.96	0.1425
OM3	12.76	0.85	11.91	6.63	3.57	2.39	5.75	0.55	2221.74	14732.05	0.1508
OM4	13.36	1.75	11.61	13.09	12.14	10.25	12.54	0.47	1899.83	13770.47	0.1380
OM5	17.39	1.52	15.87	8.74	3.07	1.33	4.21	0.40	2013.43	14479.39	0.1391
OM6	23.24	1.53	21.72	6.57	2.63	2.09	3.66	0.24	2182.36	15638.26	0.1396
OM7	18.26	0.72	17.54	3.97	7.91	5.52	1.45	0.19	2156.07	15180.96	0.1420
OM8	28.11	1.48	26.63	5.26	13.17	2.46	14.46	0.69	2154.81	15873.00	0.1358
OM9	16.25	1.14	15.11	7.00	0.16	1.60	9.15	0.35	2014.37	14405.00	0.1398
OM10	33.80	0.75	33.05	2.21	0.57	1.21	8.61	0.25	2072.74	14319.19	0.1448
ON1	6.35	0.27	6.08	4.18	0.16	0.03	0.12	0.11	2052.54	14481.15	0.1417

ON2	8.50	0.14	8.36	1.66	0.16	0.03	0.05	0.05	2217.12	15861.84	0.1398
ON3	5.09	0.35	4.74	6.89	0.43	0.37	0.07	0.04	2143.93	14754.92	0.1453
ON4	8.03	0.12	7.91	1.48	0.16	0.03	0.10	0.05	2010.42	14006.51	0.1435
ON5	7.62	0.69	6.93	9.03	0.39	0.19	0.05	0.09	2058.84	15016.90	0.1371
ON6	10.12	0.49	9.63	4.85	0.85	0.29	0.93	0.05	2278.79	16278.02	0.1400
ON7	9.91	2.50	7.41	25.25	1.00	0.19	0.05	0.14	2031.76	13585.23	0.1496
ON8	11.05	2.24	8.82	20.24	6.22	0.64	0.12	0.10	1837.92	13689.16	0.1343
ON9	10.45	0.86	9.59	8.27	0.33	0.03	0.07	0.13	2202.43	15060.12	0.1462
ON10	9.35	0.72	8.63	7.68	0.16	0.03	0.10	0.15	2234.34	15907.98	0.1405
FM1	13.69	0.00	13.68	0.02	0.45	0.37	0.07	0.13	1896.44	13616.81	0.1393
FM2	6.42	0.10	6.32	1.50	0.57	0.27	0.12	0.13	2275.75	15103.38	0.1507
FM3	16.74	0.31	16.43	1.87	0.93	0.22	0.22	0.09	1847.31	13250.98	0.1394
FM4	7.00	0.00	7.00	0.04	0.16	0.03	0.12	0.13	2204.78	15305.84	0.1440
FM5	8.84	0.31	8.53	3.54	3.03	0.29	3.79	0.14	1998.49	13633.59	0.1466
FM6	4.29	0.29	4.00	6.75	0.60	0.34	0.10	0.08	1986.60	13825.52	0.1437
FM7	18.53	2.28	16.26	12.28	10.89	0.93	7.97	0.94	1801.06	13871.26	0.1298
FM8	32.40	0.14	32.26	0.43	0.16	0.22	0.10	0.13	2087.62	14493.97	0.1440
FM9	14.33	0.06	14.27	0.44	0.60	0.24	0.07	0.09	2037.52	13883.84	0.1468
FM10	65.91	0.16	65.75	0.24	0.16	0.44	0.05	0.07	1740.87	11008.86	0.1581

FN1	9.83	1.25	8.58	12.70	7.47	0.96	8.86	0.14	2097.42	14847.47	0.1413
FN2	4.02	0.41	3.61	10.10	0.70	0.19	0.12	0.08	2128.23	14776.50	0.1440
FN3	4.44	0.66	3.78	14.80	0.79	0.03	0.17	0.05	2076.57	14794.45	0.1404
FN4	3.90	0.11	3.78	2.84	7.51	0.42	1.01	0.06	1933.78	13808.02	0.1400
FN5	10.33	0.62	9.71	6.01	5.98	0.37	0.81	0.12	1519.43	10931.32	0.1390
FN6	6.95	0.06	6.89	0.80	0.91	0.22	0.17	0.12	2204.85	15537.73	0.1419
FN7	9.23	0.70	8.53	7.56	3.98	0.17	0.61	0.08	2059.70	14319.87	0.1438
FN8	11.28	1.11	10.17	9.85	12.87	0.22	1.70	0.09	2034.30	13480.50	0.1509
FN9	14.49	1.21	13.28	8.37	17.60	4.22	20.47	0.17	1947.55	13605.86	0.1431
FN10	17.38	1.47	15.91	8.44	2.62	0.49	3.62	0.12	1945.92	13748.01	0.1415
NM1	12.40	0.96	11.44	7.74	9.03	4.39	12.18	0.10	2220.61	15813.49	0.1404
NM2	14.46	0.67	13.78	4.66	0.46	0.19	0.10	0.07	1978.34	13752.69	0.1439
NM3	12.99	0.00	12.99	0.02	0.16	0.03	0.10	0.08	2120.30	14959.85	0.1417
NM4	10.19	0.92	9.27	9.07	2.20	0.76	3.25	0.10	1992.69	14033.78	0.1420
NM5	12.79	0.88	11.91	6.90	15.03	2.76	18.10	0.12	2041.63	14118.60	0.1446
NM6	13.19	0.79	12.40	5.99	0.54	0.22	0.07	0.07	2068.28	13687.56	0.1511
NM7	10.95	0.19	10.76	1.78	0.45	0.27	0.10	0.09	1992.40	13654.40	0.1459
NM8	12.89	0.37	12.53	2.83	0.37	0.03	0.12	0.10	2006.26	13834.95	0.1450
NM9	25.96	5.91	20.05	22.78	8.20	5.67	11.02	0.25	2257.58	16033.59	0.1408

NM10	26.22	0.13	26.08	0.50	4.53	2.88	0.69	0.12	2002.60	14173.11	0.1413
NN1	14.90	0.07	14.83	0.45	0.16	0.07	0.12	0.05	1785.86	10822.03	0.1650
NN2	14.39	0.20	14.18	1.42	0.81	0.12	0.10	0.10	1813.90	12427.92	0.1460
NN3	21.00	0.24	20.76	1.12	0.39	0.22	0.12	0.07	2033.70	13446.21	0.1512
NN4	11.87	0.00	11.86	0.02	0.16	0.03	0.07	0.06	1875.48	13300.03	0.1410
NN5	4.55	0.00	4.55	0.06	0.34	0.03	0.10	0.09	1947.88	12468.72	0.1562
NN6	10.65	0.00	10.65	0.03	0.16	0.03	0.17	0.07	2090.62	12740.82	0.1641
NN7	12.27	0.00	12.26	0.02	0.39	0.26	0.10	0.10	1965.16	13065.64	0.1504
NN8	21.65	0.00	21.64	0.01	0.61	0.19	0.15	0.13	1940.72	13007.39	0.1492
NN9	24.78	0.23	24.55	0.92	0.64	0.24	0.24	0.11	1778.20	12872.61	0.1381
NN10	36.00	0.08	35.91	0.23	0.61	0.24	0.15	0.09	1708.64	12253.62	0.1394

Sample	Partition Coefficients				
	log KD THg	log KD Hg(II)	log KD MeHg	log KD Fe	1/sqrt(KD MeHg)
OM1	4.09	4.10	3.88	5.39	0.01
OM2	4.10	4.18	2.91	4.07	0.03
OM3	4.00	4.02	3.32	4.04	0.02
OM4	3.96	4.01	2.66	3.61	0.05
OM5	3.84	3.88	2.99	4.13	0.03
OM6	3.69	3.72	2.84	4.16	0.04
OM7	3.85	3.86	2.88	3.70	0.04
OM8	3.72	3.73	3.25	3.48	0.02
OM9	3.86	3.89	3.23	5.37	0.02
OM10	3.50	3.51	3.27	4.85	0.02
ON1	4.14	4.16	3.21	5.41	0.02
ON2	3.91	3.92	3.59	5.44	0.02
ON3	4.32	4.35	3.26	5.04	0.02
ON4	4.09	4.10	3.15	5.38	0.03
ON5	4.10	4.14	3.24	5.05	0.02
ON6	4.01	4.03	3.16	4.72	0.03
ON7	4.02	4.14	3.09	4.62	0.03
ON8	3.97	4.06	2.62	3.83	0.05
ON9	3.93	3.96	3.07	5.11	0.03
ON10	4.01	4.03	3.20	5.42	0.03
FM1	3.89	3.95	5.48	5.02	0.00
FM2	4.20	4.20	3.93	4.92	0.01
FM3	3.96	3.81	3.55	4.71	0.02
FM4	4.23	4.33	5.46	5.48	0.00
FM5	4.22	4.10	3.39	4.17	0.02
FM6	4.40	4.40	3.34	4.88	0.02
FM7	3.93	3.52	3.52	3.62	0.02
FM8	3.72	3.44	3.55	5.46	0.02
FM9	4.09	4.03	4.05	4.83	0.01
FM10	3.34	2.84	3.57	5.48	0.02
FN1	3.94	4.11	3.08	3.69	0.03
FN2	4.52	4.56	3.30	4.81	0.02
FN3	4.53	4.56	3.11	4.74	0.03
FN4	4.61	4.62	3.96	4.04	0.01
FN5	4.22	4.19	3.03	3.85	0.03
FN6	4.31	4.30	4.12	4.67	0.01
FN7	4.22	4.24	2.88	4.04	0.04
FN8	4.11	4.17	3.18	3.59	0.03

FN9	4.14	4.07	3.17	3.43	0.03
FN10	3.99	4.10	2.94	4.26	0.03
NM1	4.00	3.96	3.12	3.67	0.03
NM2	3.85	3.86	3.36	4.96	0.02
NM3	3.92	4.07	5.45	5.47	0.00
NM4	4.17	4.10	2.81	4.29	0.04
NM5	3.92	4.09	3.17	3.45	0.03
NM6	3.88	3.93	3.08	4.90	0.03
NM7	3.75	4.17	3.59	4.96	0.02
NM8	3.84	4.13	3.27	5.02	0.02
NM9	3.77	3.93	2.64	3.72	0.05
NM10	3.24	3.73	3.86	3.94	0.01
NN1	3.87	3.75	4.02	5.50	0.01
NN2	3.97	3.97	3.84	4.80	0.01
NN3	3.82	3.86	3.65	5.10	0.02
NN4	4.12	4.13	5.45	5.49	0.00
NN5	4.52	4.58	5.46	5.23	0.00
NN6	4.11	4.12	5.62	5.52	0.00
NN7	4.09	4.09	5.57	5.17	0.00
NN8	3.84	3.82	5.69	4.92	0.00
NN9	3.81	3.91	3.74	4.96	0.01
NN10	3.75	3.67	4.05	4.95	0.01